COMPARISON OF AUTONOMIC FUNCTIONS IN PERIMENOPAUSAL WOMEN WITH POSTMENOPAUSAL WOMEN

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CERTIFICATE

This is to certify that this dissertation entitled **"COMPARISON OF AUTONOMIC FUNCTIONS IN PERIMENOPAUSAL WOMEN WITH POSTMENOPAUSAL WOMEN"** is a bonafide work done by DR.M.VIJAYALAKSHMI, under my guidance and supervision in the Department of Physiology, Thanjavur Medical College, Thanjavur during her post graduate course from 2010 to 2013.

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DECLARATION

I solemnly declare that this dissertation **"COMPARISON OF AUTONOMIC FUNCTIONS IN PERIMENOPAUSAL WOMEN WITH POSTMENOPAUSAL WOMEN"** was done by me in the Department of Physiology, Thanjavur Medical College and Hospital, Thanjavur under the guidance and supervision of my Professor Dr.R.VINODHA, M.D., Department of Physiology, Thanjavur Medical College, Thanjavur between 2010 and 2013.

This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai in partial fulfillment of University requirements for the award of M.D Degree (Branch – V) in Physiology.

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CONTENTS

INTRODUCTION

INTRODUCTION

One of the significant demographic changes noticed both in the developed and developing countries is the gradual increase in the ageing population [(1)]. The average lifespan of a women in India is 65 years while in developed countries it is 80 years, so women of our country deserve special attention[(2)]. A women, gradually transcends into perimenopause and after a couple of years into menopause in her reproductive period [(3)].

Perimenopause includes the period beginning with the first clinical, biological and endocrinological features of the approaching menopause such as vasomotor symptoms, menstrual irregularities and ends 12 months after the final menstrual period[(2)]. It refers to the time period in the late reproductive years usually late forty's to early fifty's.

Menopause, consists of gradual transition from reproductive to the non-reproductive phase of life, which is a normal ageing phenomenon in women[(4)].The term menopause refers to a point in time that follows one year after the cessation of menstruation[(5)]. The postmenopause describes those years following this point and this occurs between the age group of 45 and 60.

Menopause is an example of endocrinal senescence. It is a normal biological event and it signifies the depletion of functional ovarian follicles that are responsible for estradiol production. Metabolic and physiological functions within the women's body are affected due to hormonal deficiency including the cardiovascular system [(6)].

With the advancement of age and menopausal duration, there are also changes in functions of different organs of the body and responses of the autonomic nervous system.

Menopause transition is the period before the final menstrual period when variability occurs in the menstrual cycles. Vasomotor symptoms like hot flushes and night sweats are the common clinical symptoms during this transition period. These symptoms suggest an alteration of either local control of blood flow to skin or the cardiovascular reflexes, which is due to alteration of autonomic hemodynamic control.

In our country, postmenopausal women are at risk of developing cardiovascular disease associated with autonomic nerve dysfunctions [(7)]. During menopausal period, alterations in autonomic nerve functions may occur and it commonly affects cardiac vagal control which is associated with sympathetic over activity [(8)]. Recent attention on postmenopausal health has led to study the autonomic function status in postmenopausal women and the relationships of decreased level of estrogen with autonomic nerve dysfunction[(9)].

Thus, this study was done to compare the autonomic functions in perimenopausal and postmenopausal women to assess any significant deviations in sympathetic and parasympathetic activity and also to test the estrogen role in the modulation of autonomic functions.

AIMS AND OBJECTIVES

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1. The study is to compare the autonomic functions in perimenopausal women with the postmenopausal women.

2. To test the hypothesis that estrogen exerts regulatory influence on autonomic nervous system in postmenopausal women.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term menopause is derived from the Greek **menos** (months) and **pausos** (ending). An average Indian woman now lives up to 65 years of age, whereas in developed countries, a lifespan up to 80 years is possible with the consequence that a woman spends one third of her life after menopause and possess health problems. The health problems cropping during this period are related to estrogen deficiency [10].

DEFINITIONS:

Premenopause:

It refers to the entire reproductive period from menarche to the final menstrual period.

Perimenopause:

Includes the period beginning with the first clinical, biological and endocrinological features of the approaching menopause such as vasomotor symptoms and menstrual irregularity and ends 12 months after the final menstrual period.

Menopause:

The permanent cessation of menstruation at the end of reproductive life due to loss of ovarian follicular activity and is recognized to have occurred after 12 consecutive months of amenorrhea, for which no pathological cause should be present.

Postmenopause:

This is from the final menstrual period, regardless of whether the menopause was induced or spontaneous.

Progressive loss of ovarian follicular units occurs throughout life. Approximately 6 to 7 million germ cells are present in the two ovaries of the developing female fetus at 20 weeks gestation. At birth, only 1 to 2 million follicular units remains in the ovaries. A more rapid depletion of follicles starts in the late 30's and early 40's and continues until a point at which the menopausal ovary is virtually devoid of follicles[(5)]. The process of atresia of the non-dominant cohort of follicles, largely independent of menstrual cyclicity, is the prime event that leads to the eventual loss of ovarian activity and menopause.

HYPOTHALAMUS –PITUITARY-OVARIAN-AXIS :

Gonadotrophin releasing hormone is released by the arcuate nucleus of the hypothalamus in a pulsatile manner, during the reproductive period. It binds with GnRH receptors on the pituitary to stimulate the luteinizing hormone and follicle stimulating hormone to release. And in turn, it also stimulates the production of the ovarian steroids: estrogen, progesterone and also inhibin. Estrogen and progesterone exerts positive and negative feedback on gonadotrophin secretion.

The loss of functional ovarian follicles during menopause, leads to the decreased production of estrogen which reduces the negative feedback to the

anterior pituitary, in turn leads to increased level of FSH. Diminished inhibin production by the ageing ovary may also contribute to the sharp rise in the FSH, a level that occurs in the perimenopausal period of life. During menopause, anterior pituitary still secretes FSH and LH in pulses, presumably after cyclic release of GnRH from the hypothalamus [10].

MENOPAUSAL SYMPTOMS:

Effects can range from discomfort to long term changes that can have a profound effect on a women's health.

Short term effects:

Amenorrhea:

Amenorrhea is defined as absence of menstruation for more than ninety days in a woman with the regular menstrual cycles.

Hot flushes:

The symptom associated with estrogen deficiency is the hot flush, also known as hot flash. This symptom is described as "recurrent, transient periods of flushing, sweating and a sensation of heat, often accompanied by palpitations, feelings of anxiety and sometimes followed by chills.

This change is particularly marked in the fingers and toes, where skin temperature can increase $10^{o}C$ to $15^{o}C$. Heart rate and skin blood flow peaks within 3 minutes of the onset of the hot flush.

Physiologically, hot flushes are due to increase in the frequency and intensity of the GnRH pulses from the hypothalamus. Some neurotransmitters also play a role on hot flushes, e.g., nor-epinephrine, serotonin, dopamine, β endorphin [11, 12].

Sleep disturbances and fatigue:

Sleep disruption is a common complaint of women with hot flushes. Disturbed sleep can lead to fatigue, irritability, depressive symptoms, cognitive dysfunction and impairment in daily functioning. Associated somatic symptoms are headache, joint pain.

Long term effects:

Low estrogen levels have a cumulative effect on many tissues. Prolonged estrogen deficiency may contribute to the development of life threatening and irreversible conditions such as cardiovascular disease and osteoporosis [11].

ESTROGEN:

Estrogens are secreted in sufficient quantities by the theca interna and granulosa cells of the ovarian follicles, in the non pregnant female. Minute quantities are also secreted by the adrenal cortices. The principal estrogen secreted by the ovaries is beta estradiol [13].

Estrogen is a steroid hormone, synthesized from cholesterol derived from blood and to a slight extent from acetyl co-enzyme A. During

synthesis progesterone and testosterone are synthesized first and then these are converted into estrogens. This reaction is stimulated by Gonadotrophin. Estrogen was chemically synthesized in the 1920s.In 1935, Mazer and Israel was the first one to report, the successful treatment for the menopausal women, who complained of vasomotor symptoms with estrogen [12].

Analysis of Estrogen:

Method: CLIA – Chemiluminescence immunoassay

A Chemiluminescence reaction is a chemical reaction in which one of the products of the reaction is light. The enzyme peroxidase can react with molecules such as luminal to yield light as part of the reaction product [14] .

The advantage of this technique is that it can be very sensitive. This method is capable of measuring low concentrations of estradiol in postmenopausal women [15].

NORMAL VALUES OF ESTROGEN DURING [16]

Premenopause	:	40-400µg/ml
Perimenopausal	:	120-160µg/ml
Menopause	:	10-20µg/ml

EFFECTS OF ESTROGEN ON AUTONOMIC FUNCTION:

Menopausal cause is due to 'burning out' of the ovaries, which results in the reduction of estrogen.

Menopause causes an increase in sympathetic activation due to endothelial dysfunction. Sympathetic activation may result in increased release of renin and angiotensin II. Endothelial dysfunction also causes reduction in Nitric oxide and increase in endothelin. Thus, there is increase in levels of vasoconstrictors (angiotensin II, endothelin) and a reduction in nitric oxide during menopause.

Estrogens, by inhibiting the angiotensin II production would increase the effects of nitric oxide. They also increase the production of prostacycline; endothelium derived hyperpolarizing factor and nitric oxide release and inhibits the degradation of bradykinin by ACE inhibition. Thus, there is a balance between the effects of angiotensin and nitric oxide which, plays an important role in the regulation of vascular tone [(17)].

Estrogens also modulate sympathetic control of smooth muscle function. It inhibits the atherosclerotic plaque formation. Catechol estrogens are formed in the central nervous system and are present at significant levels in the pituitary and hypothalamus. They may serve as neurotransmitters and interact with adrenergic as well as steroid receptors. It also inhibits proliferation of smooth muscle cells [(18)].

Sympathetic and Parasympathetic system controls the cardiovascular function and the risk of cardiovascular disease was enhanced by abnormal autonomic activity. Several studies also showed that the cardiovascular function is modulated by estrogen.

Through feedback inhibition, estrogen was found to inhibit the activity of tyrosine hydroxylase, which is a rate limiting enzyme in catecholamine synthesis

During sympathetic nerve activation, release of nor-adrenaline is modulated by presynaptic control mechanisms. The feedback inhibition, which was mediated by nor-adrenaline stimulation of presynaptic α_2 adrenoceptors, is the most important one. Estrogen also increases the density and promotes the function of α2 adrenoceptors. The reduction in maximum levels of heart rate and systolic blood pressure was due to decrease in catecholamine response by estrogen [(19)].

In the central nervous system, estrogen potentiates cholinomuscarinic activity. Choline uptake is facilitated by estrogen due to high affinity uptake systems. Estrogen is responsible for the neural development and its functions. These types of neuronal actions of estrogen are also reported in other neurotransmitters including Dopamine, Serotonin, and Nitric oxide. Estrogens also affects the synthesis and actions of angiotensin II and Nitric oxide in the central nervous system and cardiovascular system, which exerts indirect modulation on autonomic neurotransmission.

AUTONOMIC NERVOUS SYSTEM:

HISTORY:

During the Roman period, **Galen** described about the sympathetic ganglion and the rami communicantes. He said that this ganglion was originated in the central nervous system (brain). The ganglion chain arised and descended from the brain especially from posterior fossa, which was hypothesized by Thomas Willis in 1664. It was associated with the involuntary action. During 1732, **Winslow** introduced the new term sympathetic nerve. Lastly, in the 19th century **Walter Gaskell** concluded that autonomic nervous system was consisted of two subdivisions. During this century further refinements were contributed by ThomasElliot**, Walter Dixon and Otto Leewi**[20].

The Autonomic nervous system is the part of the nervous system that is responsible for homeostasis. The word autonomic is derived from the Greek word the **auto** means "self**"** and nomos means "**control.**" It is also called vegetative system, because it controls the vegetative functions. This system helps to control arterial pressure, gastrointestinal secretion, and gastrointestinal motility, emptying of urinary bladder, sweating, body temperature, and also other activities. One of the most important features of autonomic nervous system is the rapidity and intensity by which it can change visceral functions. Within 3 to 5 seconds it can increase the heart rate to twice the normal and within 10 to 15 seconds the arterial pressure can be doubled or it can be decreased low enough within 10 to 15 seconds to cause fainting [21].

ANATOMY AND PHYSIOLOGY OF AUTONOMIC NERVOUS SYSTEM:

Autonomic nervous system has two main physiological as well as anatomical divisions, sympathetic and parasympathetic, each having a central and peripheral component.

SYMPATHETIC NERVOUS SYSTEM:

Sympathetic division also called thoracolumbar division consists of thoracic and lumbar chains of sympathetic ganglia.

Preganglionic neurons:

The cell bodies of sympathetic Preganglionic neurons are found in the intermediolateral horn of the spinal cord from level T1 to L2. Hence sympathetic division is also known as thoracolumbar outflow of autonomic nervous system. From the spinal cord the axons of visceral motor neurons leave, via ventral root and then travel through the white rami communicantes to the paravertebral ganglia of the sympathetic trunk[22].

Some fibers may go up or down in the sympathetic trunk to terminate in the ganglia located at a higher or lower level. Other fibers may travel through the sympathetic trunk and exit without synapsing via splanchnic nerve and terminate in a prevertebral ganglion.

Sympathetic ganglia:

Sympathetic ganglia are of two types:

1. Paravertebral ganglia
2. Prevertebral or collateral ganglia
3. Peripheral or terminal ganglia

Paravertebral ganglia:

Paravertebral ganglia are arranged as enlargement along the entire length of two sympathetic trunks. They are divided into:

Cervical ganglia: These are three in number – superior, middle and inferior.

Thoracic ganglia: These are 11-12 in number

Lumbar ganglia: are 4 in number

Prevertebral ganglia:

Are three in number- celiac ganglion, inferior mesenteric ganglion, and superior mesenteric ganglion.

Peripheral ganglia:

Are located within or close to structures innervated by them. Heart, bronchi, pancreas, and urinary bladder are innervated by the terminal ganglia.

Postganglionic neurons:

Sympathetic postganglionic neurons are located primarily in ganglia on the sympathetic trunks. The axons may pass through a grey ramus communicantes and re-enter ventral root to reach a spinal nerve. These fibers are unmyelinated.

Sympathetic afferent fibers:

The afferent myelinated fibres from the viscera pass through the sympathetic ganglia without synapsing anywhere. These fibers enter the spinal nerve via the white rami communicantes. It reaches their cell bodies in the posterior root ganglia of the spinal nerve. After entering the spinal cord, it forms the afferent limb of the local reflex arc. Some also travel up to higher autonomic centers in the brain [(22)].

PARASYMPATHETIC NERVOUS SYSTEM:

The parasympathetic fibers form the craniosacral outflow, consisting of cranial parasympathetic outflow and sacral parasympathetic outflow.

Cranial component:

Cell bodies of Preganglionic neurons of cranial component of parasympathetic system are located in the brainstem. Brainstem parasympathetic neurons innervate structures in the head, neck, thorax and

abdomen. Parasympathetic axons from brainstem travel in III, VII, IX and X. Nuclei of the cranial nerves are present in the midbrain in tectum, Pons and medulla. Therefore, these nuclei serve as the centers for the integration of autonomic reflexes for the organ systems they innervate.

Sacral component:

Sacral parasympathetic neurons innervate structures in the pelvis.

Preganglionic neurons:

Cell bodies are found in the intermediolateral grey horn of sacral segments S2, S3, S4 of spinal cord. Their axons form, Preganglionic fibers which pass out through the ventral spinal root of corresponding nerves. After leaving from the spinal nerves, it forms pelvic splanchnic nerves, which finally ends in pelvic autonomic plexus.

Postganglionic neurons:

These neurons are located in the pelvic autonomic plexuses close to or within the viscera. Their axons run a very short course to supply the concerned pelvic viscera. The ratio of Preganglionic fibers to postganglionic fibers is 1:1.

Thus, the action of the parasympathetic system were found to be more discrete and localized. But, in sympathetic system there is a diffuse discharge. These fibers also supply the rectum, the sigmoid colon, the descending colon and the left one- third of transverse colon [21, 22].

Parasympathetic afferent fibers:

The afferent fibers pass from viscera to their cell bodies found in the sensory ganglia of the cranial nerves or in sacrospinal nerves (posterior root). The central axons then take part in formation of local reflex arc, or it may also travel to higher centers of the autonomic nervous system.

Distinguishing Features of the Sympathetic and Parasympathetic Systems

Sympathetic system	Parasympathetic System
Originates in thoracic and lumbar regions of the spinal cord.(T1-L2)	Originates in brainstem (cranial nerves III,VII,IX,X)and sacral region of spinal cord.(S2-S4)
Ganglia are found in paravertebral sympathetic ganglion.	Terminal ganglia are merged within the target tissue
Ratio of Preganglionic fibers to postganglionic fibers is 1:20	Ratio of Preganglionic fibers to postganglionic fibers is 1:3
Cholinergic Preganglionic fibres are short; Adrenergic postganglionic fibres are long	Cholinergic Preganglionic fibres are long; cholinergic postganglionic fibres are short.
More divergence that coordinates the activity of neurons in the spinal cord.	Less divergence
Nor-epinephrine is the neurotransmitter of postganglionic neurons.	Acetylcholine is the neurotransmitter of postganglionic neurons.
Predominant action is during emergency, “fight or flight “reactions and exercise	The action is present during quiet resting conditions

HIGHER CONTROL OF AUTONOMIC NERVOUS SYSTEM:

The hypothalamus has a controlling influence on the autonomic nervous system and appears to integrate the autonomic neuroendocrine systems, thus preserving body homeostasis. Essentially the hypothalamus should be regarded as a higher nervous centre for the control of lower autonomic centers in the brainstem and spinal cord. For its major role in autonomic functions, hypothalamus was designated as "**Head ganglion of the autonomic nervous system".**

Stimulation of hypothalamus (anterior part) can influence parasympathetic responses, whereas stimulation of the posterior part of the hypothalamus gives rise to sympathetic responses. In addition lower brain stem centers such as vasopressor, vasodilator, cardioaccelarator and respiratory centre has been found in the reticular formation. The neurons of the thoracolumbar outflow of the sympathetic part of the system and neurons of the craniosacral outflow of the parasympathetic part of the system receive their control through the descending tracts of the reticular formation [(23)].

These control centres, present in the brainstem and hypothalamus are highly influenced by higher brain areas. The cerebral cortex and the limbic system also influence the autonomic nervous system activities along with emotional responses by way of hypothalamic-brainstem pathways. For example, blushing during an embarrassing moment, this originates in the frontal association cortex, results in vasodilatation of blood vessels to the face.

Other emotional responses like breaking out in a cold sweat, a racing heart rate were also influenced by these higher brain areas.

At the level of spinal cord few autonomic reflexes were also processed. They are defecation and micturition reflex. These reflexes also occur without input from the brain even though they are influenced from higher nervous centres.

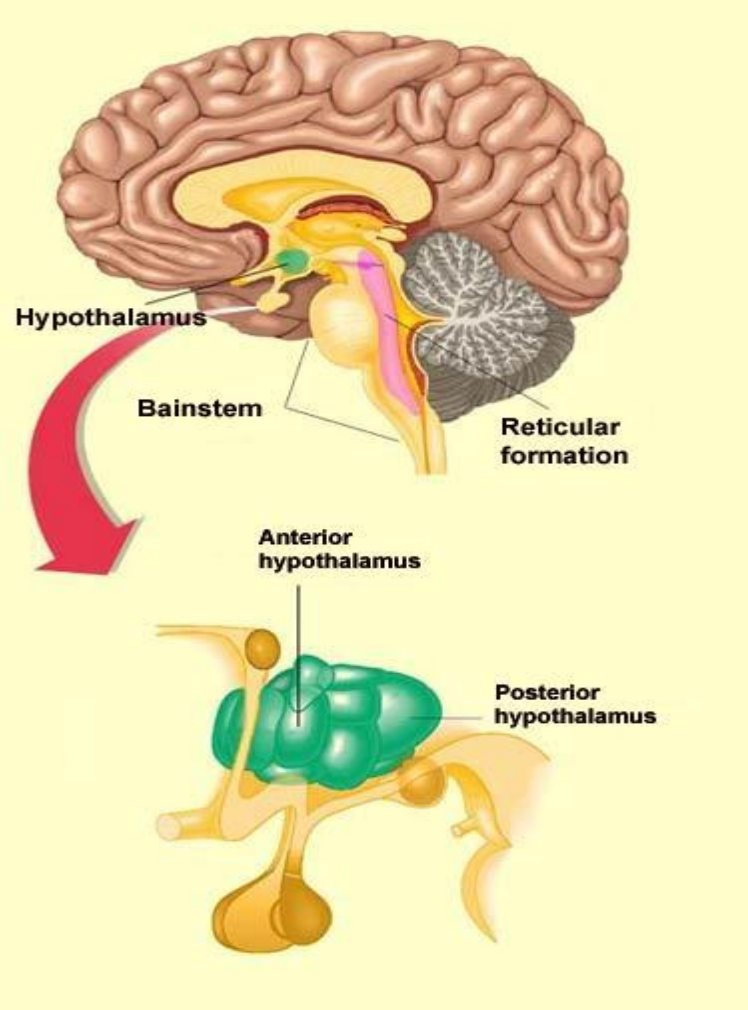


Figure No: 1 Higher control of the ANS

Neurotransmitters of the Autonomic Nervous System:

The sympathetic and parasympathetic nerve fibres secrete mainly one or the other of two synaptic transmitter substances, Acetylcholine or Nor-epinephrine. Neurotransmitters are produced in the axon varicosities and are stored in vesicles and released whenever needed. Those fibres that secrete acetylcholine are said to be cholinergic. Those that secrete nor-epinephrine are said to be adrenergic, a term derived from adrenalin, which is an alternate name for epinephrine. All Preganglionic neurons are cholinergic in both sympathetic and parasympathetic nervous system. Either all or almost all of the postganglionic neurons of the parasympathetic system are also cholinergic. Conversely, most of the postganglionic sympathetic neurons are adrenergic [23].

The cells of the adrenal medulla are considered to be the modified sympathetic postganglionic neurons. They release hormones into the blood. The hormonal output of the adrenal medulla is nearly 20% mainly the nor-epinephrine. The epinephrine is 80%. The adrenal medulla contains an enzyme that converts nor-epinephrine to form epinephrine. The formation of epinephrine, which is also known as adrenaline, is produced during stress. These two hormones are called as catecholamines that are released by the adrenal medulla. However, the postganglionic sympathetic nerve fibres to the sweat glands, to the piloerector muscles of the hairs and to a very few blood vessels are cholinergic.

The terminal nerve endings of the parasympathetic system secrete acetylcholine. Almost all of the sympathetic nerve endings secrete nor-epinephrine, but a few secrete acetylcholine. Therefore, acetylcholine is called a parasympathetic transmitter and nor-epinephrine is called a sympathetic transmitter [20-24].

Termination of Neurotransmitter activity:

Termination is necessary to allow new signals and influence effector tissue function. The first and foremost mechanism done by the cholinergic synapses is enzymatic degradation. Choline and acetate are the two products that are formed due to the hydroxylation of acetylcholine by acetylcholinesterase. Thus, acetyl cholinesterase is the fastest acting enzymes in our body and removal of acetylcholine occurs in less than 1 msec.

The reuptake of nor-epinephrine into the sympathetic nerve, that releases is the most significant mechanism for the removal of nor-epinephrine from the neuro-effector junction. Nor-epinephrine is metabolized intraneurally by Monoamine oxidase. Catechol-O-methyltransferase enzyme inactivates the circulating catecholamine like epinephrine and nor-epinephrine in the liver [23].

FUNCTIONS OF THE AUTONOMIC NERVOUS SYSTEM:

Sympathetic nervous system:

It is designed for quick, immediate and massive action and in conjunction with the adrenal medulla initiate reactions in conditions of stress.

The sympathetic-adrenal axis comes into play in conditions of emergencies and threatened danger causing fright, flight or fight. The sympathetic system that elicits the "fight or flight" reaction is a whole body response [25].

1. Cardiac output is increased due to increase in the heart rate and myocardial contractility.

2. Sympathetic activation of vascular smooth muscle causes vasoconstriction, mostly in kidneys and gastrointestinal organs. It helps to divert the blood towards the contracting muscles.

3. The movement of air inside and outside of the lungs is facilitated by bronchodilatation. Thus, the oxygen uptake from the atmosphere and the carbon dioxide elimination from the body are enhanced.

4. The concentration of glucose in the blood is increased by glycogenolysis and gluconeogenesis in the liver.

5. The fatty acid substances in the blood are increased by lipolysis in adipose tissue. For contraction, skeletal muscle utilizes these fatty acids to form metabolic energy. During the conditions of increased physical exertion, sweating is caused by the sympathetic activity that makes the individual to thermo regulate.

6. The lens adapts for distance vision by dilating the pupil and allowing more light to enter the retina.

Each Preganglionic fibre arborizes with a large number of ganglia, thus enabling a simultaneous massive reaction to be set in motion as a result of a single command. The cardiovascular, respiratory, glycogenolytic and other actions enable to meet the threatened dangers. This type of sympathetic activity involves expenditure of energy and is called "**Catabolic division** of autonomic nervous system" [23, 25].

Parasympathetic nervous system:

It has long preganglionic fibres like that of the vagus and each ganglion has connections with only limited number of postganglionic fibres, so that the action is individualized and discrete.

1. It decreases the heart rate that helps to conserve energy.

2. The swallowing of food is enhanced by salivary secretion.

3. Gastric, intestinal motility and secretion are stimulated to favour the absorption of nutrients.

4. Exocrine and endocrine secretion from the pancreas is promoted and thus contributes to the chemical breakdown of the food in the intestine. Storage of nutrient substances in the tissues are promoted by the release of insulin from the Islet's of pancreas.

5. Contraction of urinary bladder results in urination.

6. Pupil is constricted and the lens adapts for near vision.

Thus, it regulates the secretor activity of the salivary, gastrointestinal and other glands, motor activity of the gastrointestinal and urinary bladder smooth muscle activity and it's action restore's body energy reserve. It favours digestion, absorption and thus referred to as "**anabolic** nervous system" [25].

AUTONOMIC RECEPTORS:

The autonomic neurotransmitters (Acetylcholine and Nor-epinephrine) produce their effects on the organs by combining with specific protein molecules known as receptors.

I. Cholinergic receptors:

a. Nicotinic receptors

b. Muscarinic receptors

Nicotinic receptors:

Location: These receptors are located at

1. Autonomic ganglia- Transmission is mediated by N2 nicotinic receptors. They are blocked by hexamethonium.

2. Neuromuscular junction- Transmission is mediated by N1 nicotinic receptors. They are blocked by atropine.

3. Adrenal medulla

Activation: are activated by

a. Acetylcholine

b. Nicotine

Effects: Produce excitation

Muscarinic Receptors:

Location: Are located in

1. Heart

2. Smooth muscles (except vascular smooth muscle)

3. Glands

Activation by:

a. Acetylcholine
b. Muscarine

Effects:

1. Inhibitory in the heart
2. Excitatory in smooth muscles and glands

Adrenergic receptors:

On the basis of their pharmacologic properties, adrenergic receptors are of two types.

1. Alpha adrenergic receptors (α_1, α_2)

2. Beta adrenergic receptors (β_1, β_2, β_3)

Alpha$_1$ receptor:

These receptors are located in smooth muscle of skin, Gastrointestinal and bladder sphincters, Radial muscle of iris.

Effects: These receptors produce excitation.

Alpha$_2$ receptors:

These receptors are located in presynaptic terminals, platelets, fat cells, walls of the gastrointestinal tract.

Effects: Often produce inhibition

Beta$_1$ receptors: Located in SA node, AV node, Ventricular muscle of the heart.

Effects: Produce excitation

Beta$_2$ receptors: located in vascular smooth muscle of skeletal muscle, Bronchial smooth muscle, wall of the GI tract, Bladder.

Effects: Produce relaxation

Beta$_3$ receptors: Located in adipose tissue.

Effects: Lipolysis [22-25]

Physiological effects of Autonomic nervous system:

Cardiovascular system:

The **heart** is innervated by parasympathetic and sympathetic neurons. The cell bodies of parasympathetic Preganglionic neurons that innervate the heart are found in the medulla.

The sympathetic innervation of the heart is from neurons in the intermediolateral columns at the T1-T4 levels. Axons of these neurons mainly synapse with the superior, middle, and inferior cervical ganglia that are the origin of postganglionic sympathetic neurons. These axons innervate the sinuatrial and atrioventricular nodes. The conduction system and myocardial muscle fibres in the ventricles was also innervated by sympathetic neurons. In systemic circulation, the arteries and veins are mostly innervated by sympathetic neurons [(20)].

Postganglionic parasympathetic neurons release acetylcholine. It activates muscarinic receptors found in the heart, that causes reduction in heart rate and conduction. Myocardial excitability and contractility was also reduced.

Nor-epinephrine released by postganglionic sympathetic axons, acting via β adrenergic receptors, mainly increases the heart rate and the conduction. Myocardial excitability and contractility was also increased. When arterial pressure increases, the vagal neurons are mostly activated via the baroreflex. But during inspiration these are inhibited, due to the action of acetylcholine

that acts immediately and is fastly inactivated by an enzyme acetyl cholinesterase. Thus the vagus controls the heart rate on a beat to beat basis.

By activating α-adrenergic receptors, it produces vasoconstriction, when the sympathetic outflow is, to the arteries, arterioles and veins of the peripheral circulation. Sympathetic activation causes splanchnic vasoconstriction that is essential for to maintain the upright posture. Thus, loss of splanchnic vasomotor outflow is the main mechanism that causes orthostatic hypotension in autonomic failure [20].

There are variety of reflexes that controls the Autonomic outflow to the heart and blood vessels. They are mainly initiated by **arterial baroreceptors** and **chemo receptors. Arterial baroreflex** are the one which provides a negative feedback mechanism that buffers fluctuations in arterial blood pressure. Carotid sinus located in the bifurcation of the common carotid artery and the aortic arch serves as baroreceptors.

As blood pressure raises nerve endings situated in the walls of the vessels are stimulated. The afferent fibres from the carotid sinus ascend on the glossopharyngeal nerve and terminate in the nucleus of tractus salitarious. The afferent fibres from the aortic arch ascend in the vagus nerve. Connector neurons in the medulla oblongata activate the parasympathetic nucleus of the vagus which slows the heart rate. At the same time reticulospinal fires descend to the spinal cord and inhibit the Preganglionic sympathetic outflow to the heart and cutaneous arterioles. The combined effect of stimulation of the

parasympathetic action on the heart and inhibition of the sympathetic action on the heart and peripheral blood vessels reduces the rate and force of contraction of the heart and reduces the peripheral resistance of the blood vessels that leads to fall in blood pressure.

During standing, the blood pressure falls, and then the baroreceptors are "unloaded". Their afferent discharge to the NTS decreases which results in reflex sympathetic excitation and parasympathetic inhibition, that causes vasoconstriction and tachycardia [20, 26, 27].

SKIN:

Skin innervated by sympathetic neurons, plays an important role in the thermoregulation and during the expression of emotional states. T2-T3 segments of the spinal cord controls the vasomotor activity and sweating in the face is via the superior cervical ganglion. In the ears and lips sympathetic flow produces vasoconstriction but in rest of the face it predominantly produces vasodilatation.

Cold produces vasoconstriction (pallor) of the skin and piloerection (goose flesh) via α-adrenergic mechanisms. Exposure to warm causes vasodilatation and through Muscarinic receptors it produces sweating. Both vasoconstrictor and sudomotor outputs are simultaneously activated producing a cold, clammy skin during certain emotional states and hemodynamic stimuli [20].

GASTROINTESTINAL TRACT:

The entire gastrointestinal tract was innervated by the dorsal nucleus of the vagus except the proximal esophagus, distal colon and rectum. Increases in propulsive motility, relaxation of sphincters are produced by vagus. Vagus also increases the secretions of the exocrine and endocrine glands of the internal viscera like stomach, intestine, pancreas, and liver. Sympathetic outflow to the gastrointestinal tract that arises from Preganglionic neurons at the level of T1-L1 segments of the spinal cord via the splanchnic nerves is involved in certain reflexes which decrease the gut motility.

URINARY BLADDER:

Through activation of detrusor muscle and relaxation of the bladder neck by the parasympathetic nerves promotes bladder emptying. Relaxation of detrusor muscle and contraction of the bladder neck are produced by sympathetic nerves, which favours the storage of urine. The sacral somatomotor output, that arises from motor neurons of the nucleus of Onuf (S2-S4) and which is carried by the pudendal nerve, stimulates contraction of the external sphincter via nicotinic receptors. Activation of these somatomotor nerves also promotes storage of urine [(20)].

EYES:

Two functions of the eyes are controlled by the autonomic nervous system. They are pupillary opening and the focus of the lens.

Dilatation of pupil is achieved by sympathetic stimulation that contracts the meridional fibres of the Iris. Constriction of pupil is by parasympathetic stimulation that contracts the circular muscle of the Iris.

GLANDS:

Stimulation of nasal, lacrimal, salivary and many gastrointestinal glands by the parasympathetic nervous system, results in copious quantities of watery secretion. The sweat glands and apocrine glands are stimulated by the sympathetic nervous system and they do not respond to parasympathetic stimulation [(20)].

EFFECTOR ORGAN	SYMPATHETIC ACTION	PARASYMPATHETIC ACTION
HEART		
SA NODE	↑ Heart rate(β1,β2)	↓Heart rate
AV NODE	↑ Conductivity(β1,β2)	↓ Conductivity
ATRIA	↑Contractility (β1,β2)	↓ Contractility
VENTRICLES	↑Contractility (β1,β2)	↓ Contractility
BLOOD VESSELS		
CUTANEOUS	Constriction(α1)	No supply
CORONARY	Dilatation(β2) Constriction(α1,α2)	Dilatation

CEREBRAL	Constriction(α1)	Dilatation
PULMONARY	Constriction(α1) Dilatation(β2)	Dilatation
RENAL	Constriction(α1,α2) Dilatation (β1,β2)	No supply
LUNGS BRONCHIOLES	Dilatation(β2)	Constriction
GI TRACT MOTILITY SECRETION	 Decrease Inhibition	 Increase Stimulation
UTERUS	Contraction(α1) Relaxation(β2)	Variable

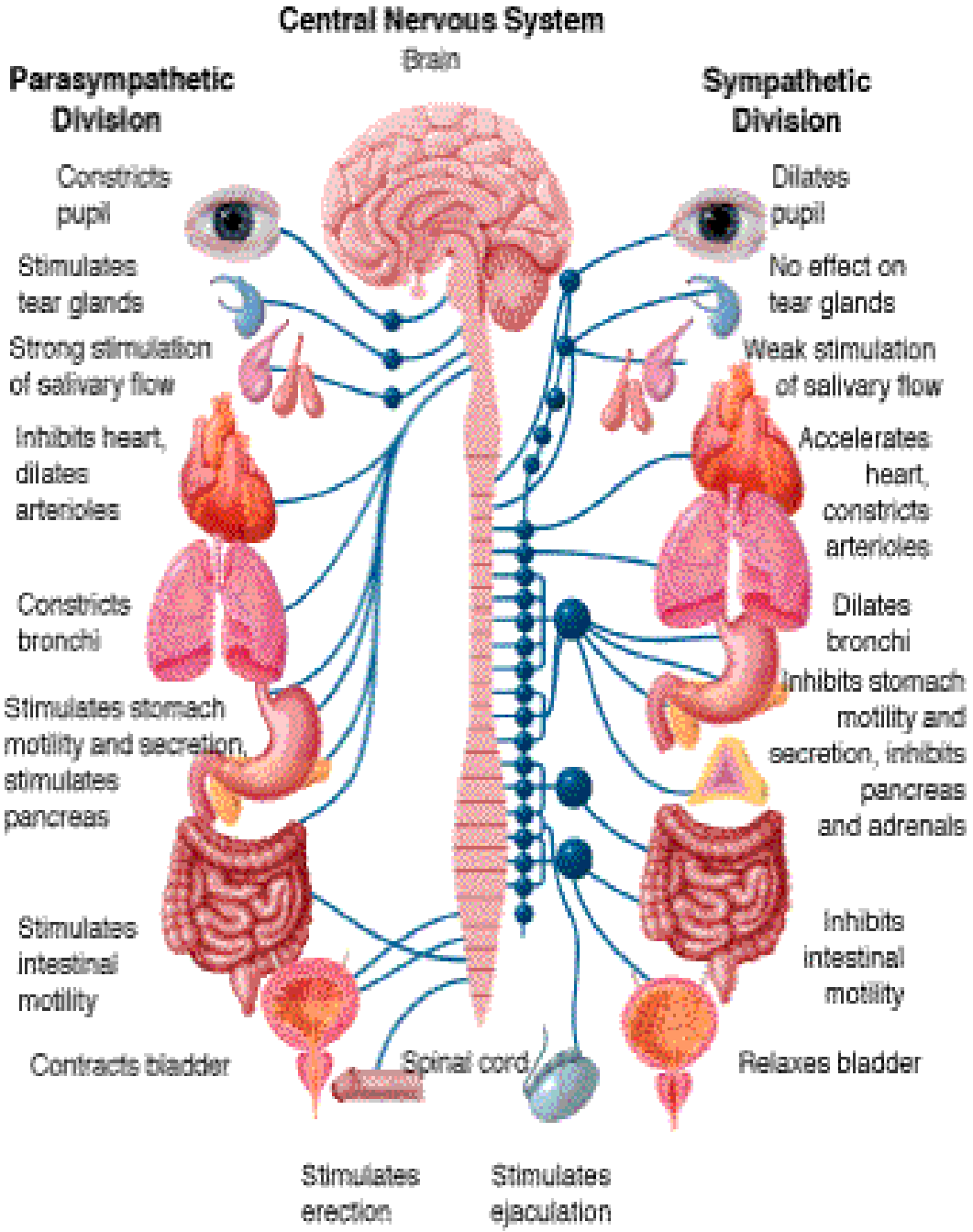


Figure No: 2 Physiological effects of ANS

AUTONOMIC FUNCTION TESTS:

A wide variety of autonomic reflex tests have been devised to assess different component of autonomic nervous system. The sympathetic

function tests are Heart rate, Blood pressure, Mean arithmetic mean, QTc interval, and Cold presser test, for assessing parasympathetic function the tests are Standing/Lying ratio, Expiration/Inspiration ratio, Valsalva ratio, 30:15 ratio.

HEART RATE and BLOOD PRESSURE:

The autonomic nervous system is the primary system for regulating heart rate in normal persons. Increase in heart rate is achieved through increase in contractile frequency via sympathetic activation. This is in part mediated through the arterial baroreflex [(28)].

Moodithaya et al., (2009) demonstrated Heart rate variability using spectral analysis and their studies showed lower heart rate variability in post menopausal women compared to that of young women. Further decline in estrogen level is associated with sympathovagal balance in postmenopausal women.

The sympathetic influence is achieved by release of epinephrine and nor-epinephrine, which causes cAMP mediated phosphorylation of membrane proteins and a resultant increase in the inward calcium current, resulting in accelerated slow diastolic depolarization. Also other endogenous factors, such as Nitric oxide, influence channel function and further modulate autonomic control of heart rate.

Latif Afrin Dill Naher et al., observed increased resting systolic and diastolic pressure in postmenopausal women, which indicates increased sympathetic activity due to low estrogen level [29].

The study done by C.L.Brockbank et al., showed reduction in the heart rate variability, associated with increase in the heart rate [30].

Brian E. Hunt et al., studied the improvement of Baroreflex activity after the administration of estrogen in the postmenopausal women [31].

ORTHOSTASIS:

Orthostatic hypotension is defined as a decrease of more than 20 mmHg in systolic pressure or a decrease of more than 10 mmHg in a diastolic pressure after raising to a standing position from a supine position. Orthostatic hypotension is an ability of the cardiopulmonary system to maintain sufficient blood pressure and adequate cerebral perfusion against gravity. Generally on rising from a supine position, an average person may lose 700ml of blood from the thorax. This results in decreased stroke volume as well as decreased systolic pressure and increased diastolic pressure. Compensation occurs via an increase in heart rate and slight peripheral vasoconstriction. Individuals intolerant to orthostasis may get venous pooling secondary to decreased muscle and vascular tone and may develop a decreased circulating blood volume in response to standing [28].

Kevin D.Monahan (2007) showed that the magnitude of the decrease in blood pressure is associated with lower levels of cardiovagal baroreflex sensitivity in older adults [32].

COLD PRESSOR TEST:

The cold pressor test is performed by the subject submerging a hand in ice cold water while blood pressure is assessed. The afferent limb is somatic, efferent limb of the reflex is sympathetic and a normal response is an elevation of blood pressure. The cold pressor test has been reported to a valuable in the assessment of baroreceptor failure and diabetic autonomic neuropathy.

Anjali Nadir Bhat (2005) showed significant variation in postmenopausal women. Their studies suggested the increased sympathetic reactivity in the postmenopausal period [4].

MEAN ARITHMETIC MEAN:

Mental stress like arithmetic, noise or emotional stress can result in increase in blood pressure and heart rate due to excessive sympathetic outflow. It is a useful measure of sympathetic efferent function.

G.nyberg et al., (1977) found that mental arithmetic stress caused increase in the heart rate and blood pressure in normotensive males and hypertensive subjects of both sexes [33].

Mi Kyong PARK et al., observed increased blood pressure of MAM in postmenopausal and perimenopausal women due to vasoconstriction caused by the sympathetic activation [34]

QTc INTERVAL:

The QT interval is an indirect measure of the duration of Ventricular depolarization and repolarization. It varies with heart rate. Thus corrected QT interval is calculated using Bazett's formula.

Arduino A.Mangoni et al demonstrated the prolongation of QTc interval in older groups. This prolongation was due to increase in vascular impedance that leads to an increase in systolic blood pressure. The increase in left ventricular wall thickness appears to be an etiological factor in the older age groups.

Their studies also showed gender related differences in which women had longer QTc values than men. This gender related differences are due to the prolongation of repolarisation duration in women and are also related to sex hormones [35].

VALSALVA RATIO:

The Valsalva maneuver occurs when intrathoracic and intra-abdominal pressure is increased during a straining procedure. The Valsalva maneuver and the cardiovascular responses that follow can be divided into four phases.

Phase I:

Phase I occurs at the onset of strain. There is transient increase in arterial blood pressure lasting for a few seconds, which is due to increased intrathoracic pressure and mechanical compression of the great vessels. The heart rate does not change in this phase.

Phase II:

Phase II occurs during straining. In the earlier part, the venous return decreases resulting in reduction of stroke volume, cardiac output, and blood pressure, which lasts about 4 seconds. In the latter part of phase I, blood pressure returns toward baseline. This recovery occurs due to increased peripheral vascular resistance as a result of sympathetic vasoconstriction. Throughout phase II, the heart rate increases steadily, which is due to vagal withdrawal in the early and increased sympathetic activity in later part of stageII.

Phase III:

Phase III recorded after the release of strain, which results in transient decrease of arterial blood pressure lasting for a few seconds.

Phase IV:

Phase IV occurs with further cessation of strain. The arterial blood pressure slowly increases and heart rate falls. Since the blood pressure rises to above and the heart rate falls below baseline level, it is called overshoot

phenomenon. It occurs following 15-20 seconds after release of strain and may last for 1minute or longer. The overshoot phenomenon is due to increase in venous return, stroke volume, and cardiac output. Valsalva ratio is the ratio of maximum heart rate in phase II to minimal heart rate in phase IV and can be calculated as the longest RR interval during phase IV to the shortest RR interval of phase II.

Naher LAD **(2009**) observed lower values of valsalva ratio in postmenopausal compared with premenopausal women. He also showed positive correlation between valsalva and serum estrogen level in postmenopausal women [(8)].

30:15 RATIO:

Normally on standing exercise reflex and mechanical effects on venous capacitance and arterial resistance vessels become operative in addition to gravitational changes. Compression of capacitance vessels by postural muscle results in displacement of blood towards the heart that increases the venous return, cardiac output, and blood pressure. During this time baroreceptors are stimulated, that reduce the sympathetic outflow, release vasoconstrictor tone, and decrease total peripheral resistance, which results in the drop of blood pressure. It last for about 6-8 seconds.

During standing heart rate increases and is continued to increase for next few seconds and then fall to a maximum by 20 secs. Parasympathetic withdrawal is the main reason for the heart rate changes.

G.V.Lathadevi et al (2011) showed slight difference in this test when compared with perimenopausal women and postmenopausal women; in which 'p' value is insignificant indicating reduced parasympathetic activity in postmenopausal women [3].

EXPIRATION: INSPIRATION:

The study of heart rate variation with respiration is indicated for testing the integrity of sympathetic cholinergic functions. The variation of heart rate with respiration is known as sinus arrhythmia, which is generated by autonomic reflexes. This phenomenon is primarily mediated by vagal innervations of heart.

Naher LAD (2009) showed lower value of heart rate response to deep breathing in postmenopausal women when compared to postmenopausal women [8].

AUTONOMIC DYSFUNCTION:

We have found major advances in recognizing, investigating and treating the autonomic disorders. Advances in non-invasive technology have resulted in more accurate pathophysiological mechanisims that enables the targeted therapy for autonomic dysfunction.

Dysautonomias refer to any dysfunction of the autonomic nervous system, may be central, peripheral or secondary to other disease processes. The autonomic nervous system has craniosacral parasympathetic and thoracolumbar

sympathetic pathways and supplies every organ in the body. This system controls the vital functions especially the arterial blood pressure and body temperature. Neurotransmitters in every pathway influence Preganglionic and post ganglionic activity. Thus autonomic diseases would occur with lesions at different sites in the brain, spinal cord or periphery [36].

Classification of autonomic dysfunction:

Autonomic dysfunction may be classified into localized and generalized disorders. Localized disorders affect a particular organ. Generalized disorders mostly affect systems. They are said to be primary when the cause is not known, and is secondary when combined with a specific disease. Drugs also cause of autonomic dysfunction, due to autonomic nerve damage. Damage to the Autonomic nervous system usually causes irreversible abnormalities

It also can be classified as sympathetic and parasympathetic dysautonomias. The most common dysautonomias are those affecting the sympathetic system and may be characterized by disorders of release, function or re-uptake of the main sympathetic chemical messenger, nor-epinephrine. Furthermore, local blood flow and clearance of nor-epinephrine from the circulation may manifest as a dysautonomia.

The sympathetic dysautonomias can be generally divided into two groups, those associated with decreased function, in which orthostasis often occurs and those associated with increased outflow, in which hypertension or tachycardia may be present.

The parasympathetic dysautonomias are most often reflected as increase in parasympathetic tone and manifest as exaggerated responses to normal physiologic conditions. Typically parasympathetic tone predominates at rest in normal humans and is balanced by local and neurohormonal responses.

Clinical features:

a) Postural hypotension is caused by sympathetic adrenergic failure.

b) Sympathetic cholinergic failure causes anhydrosis.

c) Dilated pupils, atonic large bowel is caused by Parasympathetic failure.

Cardiovascular system:

Orthostatic hypotension:

Orthostatic or postural hypotension is defined as fall in blood pressure of 20mm Hg of systolic or 10mm Hg of diastolic while standing. Orthostatic symptoms include lightheadedness, palpitations, tremulousness, visual changes, discomfort or throbbing of the head, poor concentration, tiredness, weakness and occasionally fainting [(36)].

Pathophysiology:

The potential pathophysiological causes include excessive venous pooling, a gravity dependent fluid shift, diminished plasma volume or red cell mass and dysfunction of the nor-epinephrine transporter. Genetic etiologies are also responsible. This may be due to nor-epinephrine

transporter deficiency. It derives from a unique A453p mutation yielding loss of gene function.

Gonadal hormones may affect homeostatic mechanism regulating the cardiovascular system. Another reason includes an estrogen-dependent change of the plasma volume or a direct estrogen receptor-mediated modulation of vascular reactivity. The presence of estrogen receptors in the heart, vascular smooth muscle, and hormone-mediated change in adrenoreceptor density, cAMP levels, and Nitric oxide synthase activity suggest a possible involvement in the regulation of the cardiovascular system.

Estrogen acts on endothelial cells to increase the response to acetylcholine by relaxation. It may also act directly on vascular smooth muscle.

Hypertension:

Estrogen either from endogenous or exogenous sources would likely to influence the neurogenic mechanism of blood pressure regulation. The symptoms associated are throbbing headache, palpitations [37].

Loss of baroreflex buffering mechanisms results in prolonged and exaggerated responses to a variety of tests, such as the cold pressor test.

Initial therapy over the first 72 hrs may include nitroprusside and clonidine.Chronically patient may continue to have labile hypertension and

tachycardia alternating with hypotension and bradycardia. This may be effectively treated with clonidine and methyldopa .

Estrogen Therapy:

Estrogens can be supplemented for the menopausal women to reduce the vasomotor symptoms. Various route of administering estrogens are oral, parenteral, topical, or transdermal routes [(11)].

The most commonly used oral estrogen preparation is

Conjugated equine estrogen - 0.625 to 1.25 mg daily

Transdermal preparation:

17 β estradiol - 0.025 to 0.1 mg patch applied twice daily

Parenteral preparation:

Estrone - 0.1 to 1 mg weekly

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was undertaken to compare the Autonomic functions in the perimenopausal women with postmenopausal women. Prospective non-interventional type of study was done. The study period extended from may 2011-2012. The subjects were selected from Thanjavur Medical College Hospital and Raja Mirasudar Hospital, Thanjavur. This study was performed on healthy women of age 45-55 yrs of perimenopausal and postmenopausal women.

Perimenopausal period refers to a period, in which there would be declining of ovarian functions with menstrual irregularities, vasomotor symptoms that ends 12 months after the final menstrual period.

Menopause: The permanent cessation of menstrual cycle for a period of one year or more.

The nature of study was explained to all the subjects. Informed written consent was obtained from all the participants. The experimental protocol was approved by the Ethical committee.

INCLUSION CRITERIA:

Healthy women of 45-55 yrs of age with

- irregular menstrual cycle
- cessation of menstrual cycle

- Symptoms of menopause which includes flushing, sweating.

EXCLUSION CRITERIA:

- Diabetics
- Hypertension
- Cardiovascular disorders
- Renal disorder
- Liver disorder
- Smoking
- Alcohol
- Oral pills
- Hormone replacement therapy

MATERIALS FOR THE STUDY:

1. Proforma to record the subject details and clinical examination findings.

2. Electrocardiograph

3. ECG-paper

4. ECG-jelly

5. Sphygmomanometer

6. Cotton

7. Ice cold water

8. Stethoscope

METHODOLOGY:

The study was carried out after explaining the procedure in detail and getting informed consent from the subjects. The experimental protocol was

1. A proforma with detailed history from the study subjects was filled.

2. A thorough clinical examination of the study subjects was done.

A detailed history regarding name, address, op.no, age , FMP(first menstrual period), LMP(last menstrual period), menopausal symptoms like hot flushes, sweating, past history regarding previous medical illness, family history regarding, mother's age of menopause, marital history.

A detailed general examination was carried out in the subjects including pulse rate, blood pressure, pedal edema, icterus, cyanosis, lymphadenopathy, and examination of CVS, RS, CNS, and Abdomen.

Baseline laboratory investigations were done for all the subjects including Blood sugar, hemoglobin, urine albumin and sugar.

For assessing the **Autonomic functions** the following tests were carried out:

For performing these tests ECG was recorded by a simple compact Electrocardiograph (Model No:Cardiofax, Manufactured by Medicaid systems)

SYMPATHETIC FUNCTION TESTS:

1. **PULSE RATE**: Pulse rate was taken by palpatory method

2. **BLOOD PRESSURE**:

Blood pressure was recorded in all the subjects, after 5 minutes of rest in sitting posture, either in the left or right arm, using sphygmomanometer, with the apparatus at the heart level [40]. Blood pressure was recorded by palpatory and auscultatory method.

Normal variation of Systolic Blood pressure - 100-140mmHg

Diastolic Blood pressure- 60-84mmHg

3. **ORTHOSTASIS:**

This is a simple test for assessing the integrity of sympathetic pathway. Blood pressure was recorded for the patient in supine position and immediately after standing for two minutes.

A fall in systolic blood pressure of 20mmHg or more or/and a fall in diastolic blood pressure greater than 10mmHg upon standing is abnormal.

4. **COLD PRESSOR TEST**:

The subject was asked to submerge one of her upperlimb in the ice cold water at 4^{o} C. The blood pressure was recorded at the end of 30 seconds of immersion and at the end of 60 seconds from the other arm.

A rise in systolic blood pressure greater than 20mmHg and an increase in diastolic blood pressure more than 10mmHg were considered abnormal [38].

5. **MEAN ARITHMETIC MEAN:**

It is a useful measure of sympathetic efferent function. The subject was made to solve some mathematical problem like to subtract 7 from 100 as quickly as possible and the blood pressure was recorded. The rise in blood pressure shows sympathetic overactivity [39].

6. **CORRECTED QT INTERVAL**:

QT interval was measured from the electrocardiograph and then standardized by converting it to QTc. For this Bazett's formula was used.

Normal value: 0.35 secs to 0.43 secs

PARASYMPATHETIC FUNCTION TESTS:

1. **VALSALVA MANEUVER:**

The subject was asked to sit in a stool with sphygmomanometer and ECG leads should be attached. The technique involves the subjects to blow into a mouthpiece connected to a manometer up to 40mmHg for 15 seconds and asked to stop blowing [38, 39, 40].

A continuous ECG was recorded in standard lead II during the straining period and immediately after the release for 30 seconds. The valsalva ratio was calculated as follows:

$$\text{Valsalva Ratio} = \frac{\text{Longest R-R interval after the strain}}{\text{Shortest R-R interval during the strain}}$$

A ratio of > 1.45 - normal

1.2 to 1.45 - borderline

< 1.2 - abnormal

2. **EXPIRATORY: INSPIRATORY RATIO:**

To assess the heart rate response to deep breath at 6 cycles per minute. The subject was asked to lie down comfortably in the supine position and was connected to ECG machine.

The subject was instructed to breathe deeply at a rate of six breaths/min (allowing 5 secs each for inspiration and expiration). The maximum and minimum heart rate was recorded in lead II with each respiratory cycle [41, 42]

It is calculated as:

$$\text{E: I Ratio} = \frac{\text{Mean of longest R-R interval during expiration}}{\text{Shortest R-R interval during inspiration}}$$

Normal > 1.21

Borderline 1.11 to 1.2

Abnormal < 1.10

3. **STANDING / LYING RATIO**:

To assess the heart rate response in lying posture from standing position. The subject was asked to stand quietly and then lie down without any support. A continuous ECG was recorded from 10 beats before and 20 beats after lying down. Thus, the standing /lying ratio was calculated as follows:

$$\text{S/L Ratio} = \frac{\text{Longest R-R interval during 5 beats before lying down}}{\text{Shortest R-R interval during 15 beats after lying down}}$$

Normal >1.01

4. **30:15 RATIO**:

To assess heart rate response to standing up from lying position. The subject was asked to lie down quietly on a couch and then to stand up and the heart rate was recorded in lead II ECG machine continuously during standing up period for more than 30 beats [(42)].

The 30:15 ratio was calculated as

$$30{:}15 = \frac{\text{Longest R-R interval around } 30^{th} \text{ beat}}{\text{Shortest R-R interval around } 15^{th} \text{ beat}}$$

Normal >1.04

Borderline 1.01 to 1.03

Abnormal <1.0

STATISTICAL ANALYSIS:

Statistical analysis was done by using statistical package for social sciences (SPSS) XVIII version. The results were analyzed by the student‘t’ test.

BIOCHEMICAL ANALYSIS:

The altered autonomic functions in postmenopausal women are due to decreased level of estrogen. To substantiate this, estrogen levels were determined by the method of electro-chemiluminescence immunoassay.

Figure No:3 AN ELECTROCARDIOGRAPH

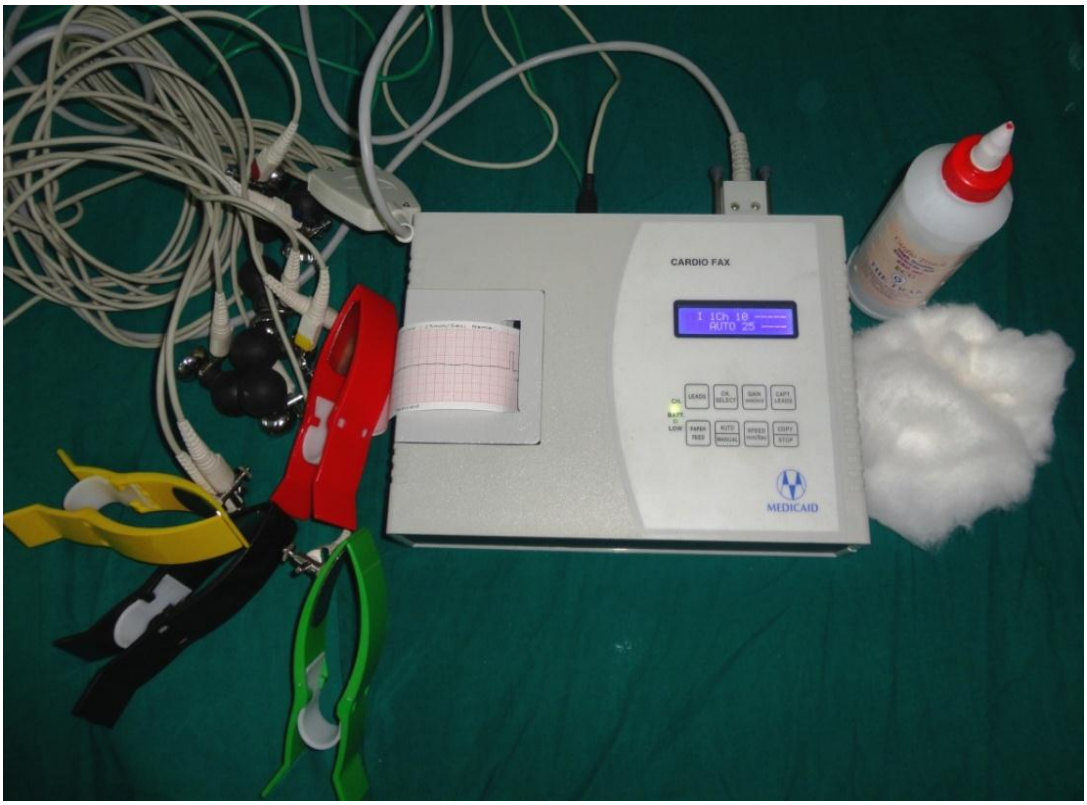


Figure No: 4. 30 : 15 Test : R-R Intervals during the 15th beat & 30th beat in Postmenopausal women.

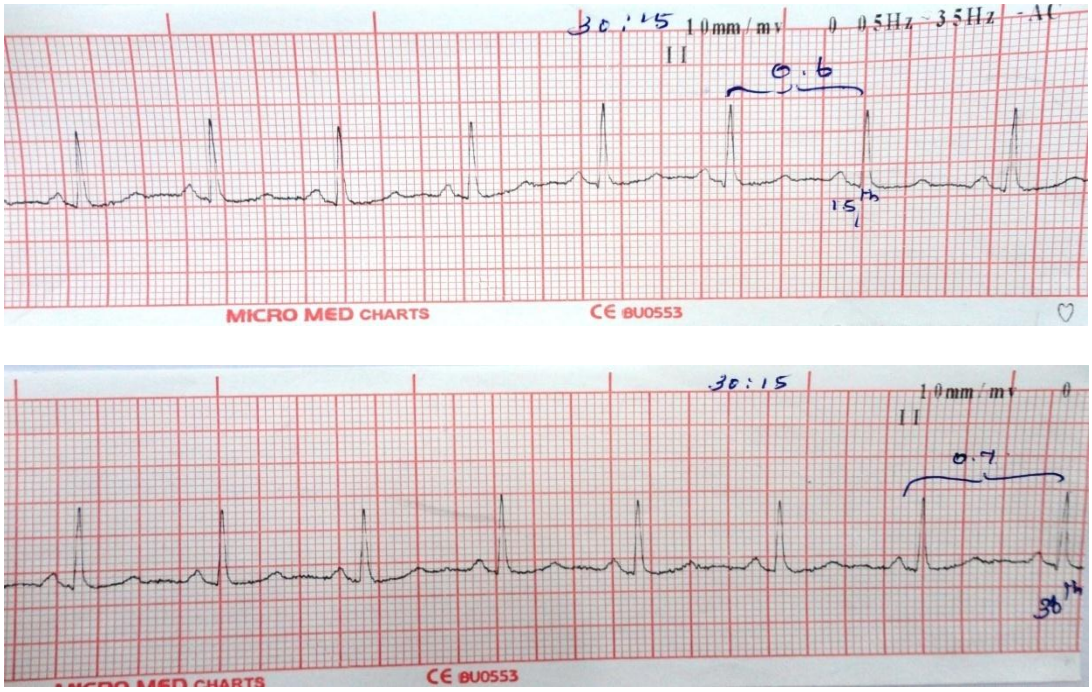


Figure No: 5. Standing / Lying Ratio : R-R intervals during standing & Lying in Postmenopausal women.

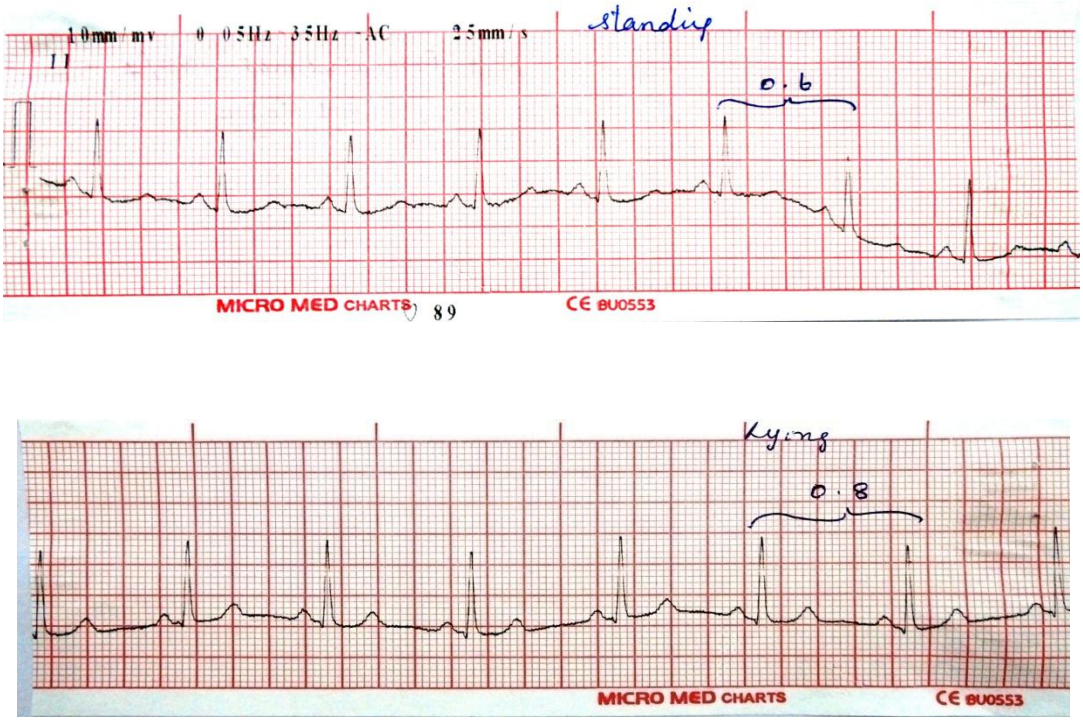


Figure No:6. Valsalva Ratio test : ECG Recordings showing R-R intervals during the strain & after the release of strain in postmenopausal women.

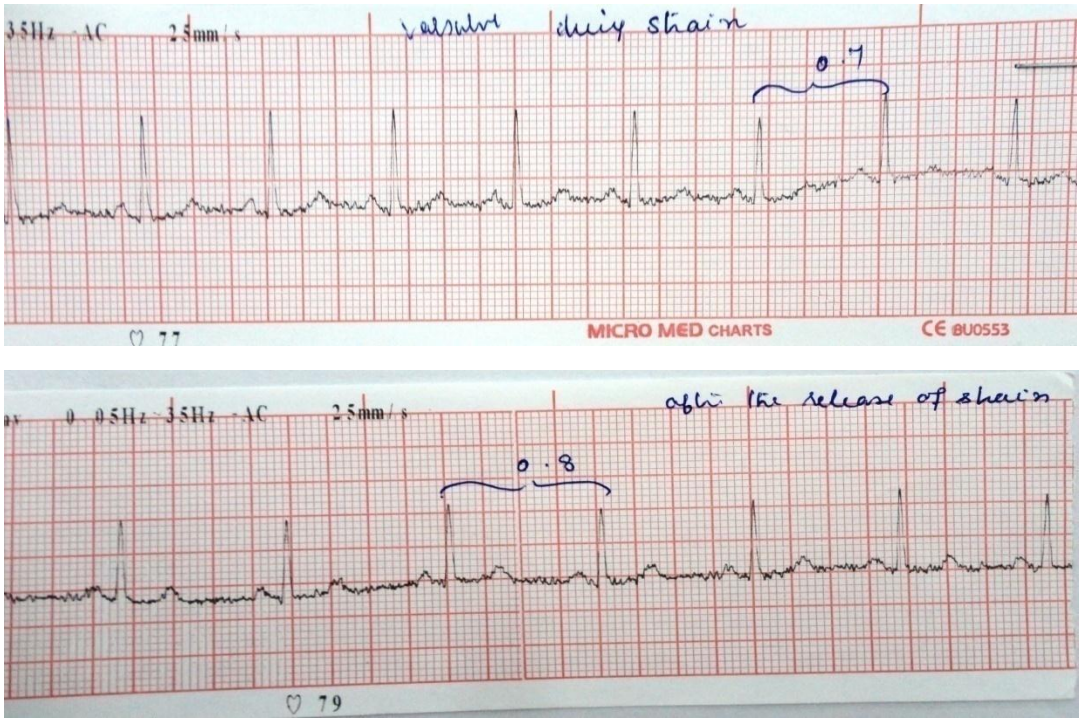


Figure No: 7. E.I Ratio : ECG recordings showing R-R intervals during Inspiration & Expiration in postmenopausal women

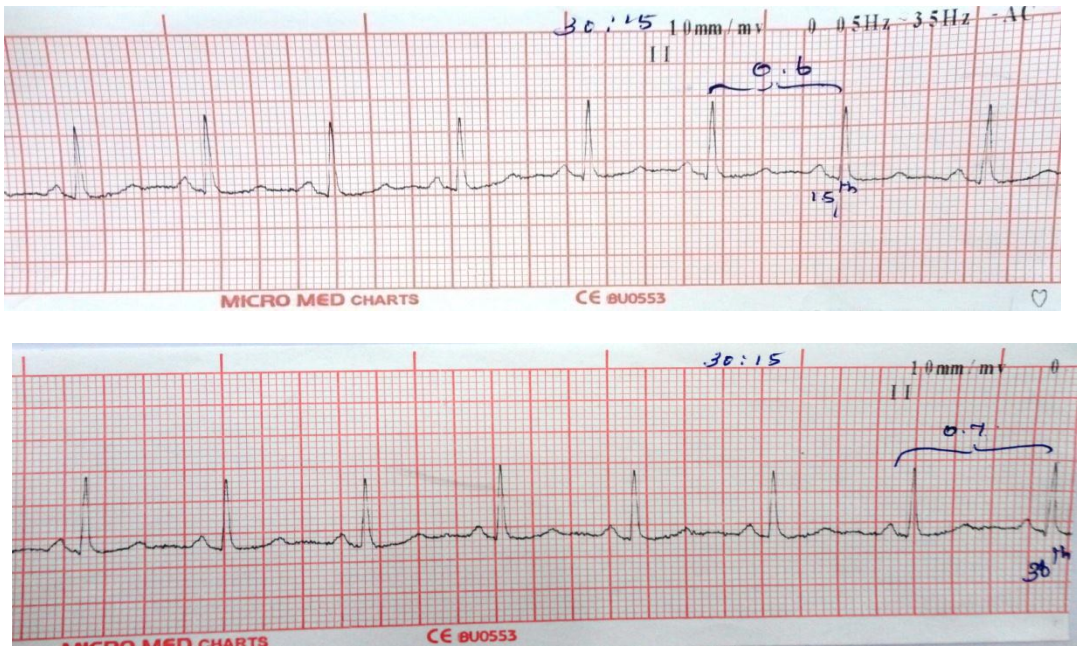


Figure No:8 CHEMILUMINESCENCE IMMUNOASSAY INSTRUMENT

Figure No:9 SERUM ESTROGEN ASSAY KIT

RESULTS

RESULTS

A prospective, non-interventional study was conducted in 80 subjects. Their age varied from 45-55 yrs.

Subjects who self reported with the H/O menstrual irregularities, irritability, sleep disturbances were included in perimenopausal group and are considered as Group A.

Subjects who self reported with H/O amenorrhea more than a year associated with vasomotor symptoms like hot flushes, night sweats were included in postmenopausal group and are considered as Group B.

The parameters evaluated in this study are sympathetic function tests which includes Pulse rate, Blood pressure, Orthostasis, Mean arithmetic mean, Cold pressor test, QTc. Parasympathetic function tests that includes Valsalva ratio, Expiration: Inspiration ratio, Standing: Lying ratio,30:15 ratio. Serum estrogen was done for both the groups.

The mean and standard deviation values of all the parameters tested in postmenopausal women were compared with the perimenopausal women. The results were analyzed by the student's t-test. The statistical significance was considered at $P< 0.05$.

These parameters were correlated with serum estrogen level by using Pearson's correlation coefficient test.

Table No: 1

Descriptive Analysis

Item	Group A (n=40)			
	Min	**Max**	**Mean**	**S.D**
Age	45	53	49.23	2.486
PR	68	96	84.25	7.389
Supine BP SBP	100	130	114.90	9.713
Supine BP DBP	60	90	74.75	5.986
Orthostasis SBP	90	130	106.35	10.688
Orthostasis DBP	60	80	71.75	5.006
Ortho variation	0	30	8.55	11.749
MAM SBP	110	130	119.35	7.167
MAM DBP	70	90	75.25	5.986
CPT SBP	110	130	119.75	7.334
CPT DBP	70	90	78.00	6.485
QTc	.20	.50	.3560	.07292
Valsal Ra	1.03	1.32	1.2232	.07301
S:L Ra	.70	108.00	3.7233	16.91081
30:15 Ra	.80	1.20	1.0660	.07635
E:I Ra	1.00	1.37	1.2270	.09487
Estrogen pg/dl	7.56	192.05	94.3275	47.15459

The baseline characteristics of Group A are shown in the table.

Table No: 2

Descriptive Analysis

Item	Group B (n=40)			
	Min	**Max**	**Mean**	**S.D**
Age	46	55	51.83	2.809
PR	70	98	84.70	5.984
Supine SBP	100	134	120.65	7.252
Supine DBP	60	84	77.10	5.728
Orthostasis SBP	90	120	103.05	7.961
Orthostasis DBP	60	80	69.95	4.356
Ortho variation	0	30	17.30	8.259
MAM SBP	110	140	123.15	6.912
MAM DBP	70	90	78.75	4.043
CPT SBP	100	130	121.55	8.283
CPT DBP	70	90	79.75	4.797
QTc	.23	.56	.3631	.10093
Valsal Ra	.77	1.53	1.0785	.1593
S:L Ra	.63	1.20	.8838	.11401
30:15 Ra	.70	1.18	.9675	.10051
E:I Ra	.80	1.30	1.0805	.10318
Estrogen pg/dl	5.00	184.40	46.0588	46.10235

The baseline characteristics of Group B are shown in the table.

Table No. 3

Comparison of Sympathetic Functions

Parameters		Group A n=40 Mean±SD	Group B n=40 Mean±SD	P value
PR		81.4±7.456	87.4 ± 7.063	0.001*
Supine BP	SBP	114.9 ± 9.71	120.65 ± 7.25	0.004*
	DBP	74.75 ± 5.98	77.10± 5.728	0.07
Standing BP	SBP	106.35±10.68	103.05±7.961	0.02*
	DBP	71.75±5.006	61.95±4.356	0.09
Orthostatic Variation		8.55±11.75	17.30±8.259	0.001*

(* P value less than 0.05 was considered to be significant)

The mean ±SD of pulse rate for Group B was 87.4±7.063 and for Group A was 81.4±7.456. It was found to be statistically increased (P=0.001).

The mean ±SD values of Supine systolic BP for Group B and Group A were 120.65±7.25and 114.9±9.71 respectively. It was found to be statistically significant (P=0.004), that shows the increased sympathetic activity.

The mean values of Supine Diastolic BP for Group B and Group A were 77.10±5.7288and 74.75±5.98 respectively. It was found to be statistically insignificant (P=0.09).

The mean values of Orthostatic variation (Systolic BP) in Group B and Group A were 17.3±8.259 and 8.55±11.75 respectively , which was found to be statistically significant (P<0.001).

Figure No:10 Parameters reflecting the sympathetic activity

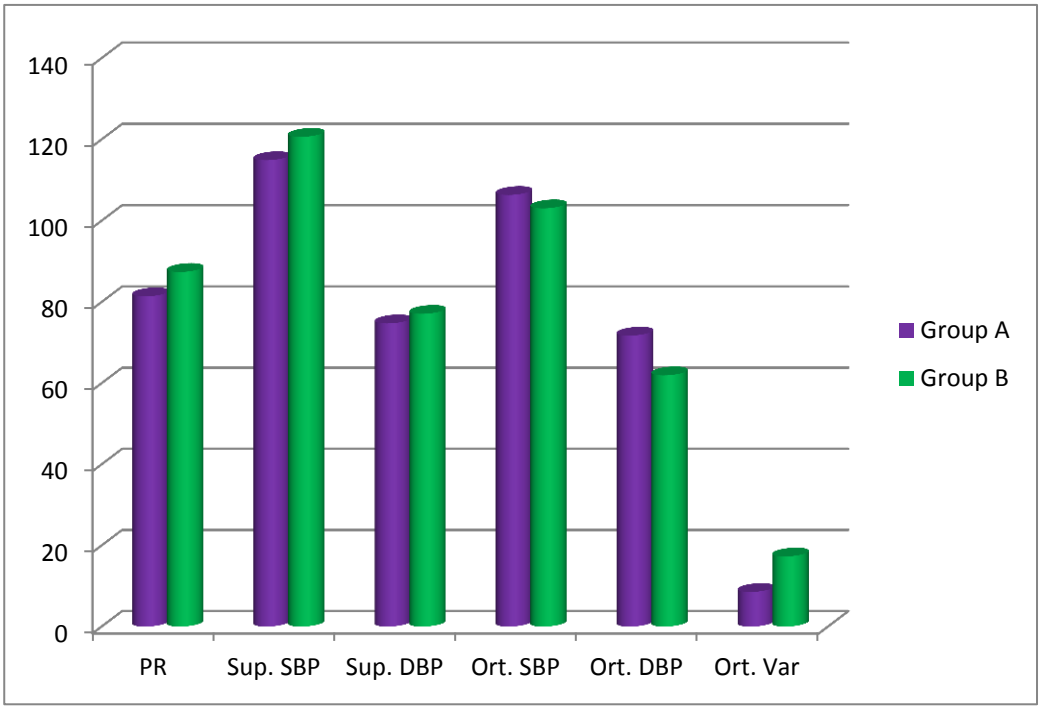


Table No:4

Comparison of Autonomic functions

Parameters		Group A n=40 mean±SD	Group B n=40 mean±SD	P value
MAM	SBP	119.35±7.16	123.15±6.91	0.018*
	DBP	75.25±5.98	78.75±4.04	0.003*
CPT	SBP	119.75±7.33	121.55±8.28	0.31
	DBP	78±6.48	79.75±4.79	0.17
QTc		0.34±0.07	0.36±0.10	0.019*

The mean value of Mental arithmetic mean(systolic BP) in Group B was 123.15±6.912 and for Group A 119.35±7.167. It was found to be significantly increased (P=0.018). The mean value of Mean arithmetic mean(Diastolic BP) in Group B and Group A were 78.75±4.043 and 75.25±5.986 respectively.The P value was 0.003, which was found to be statistically significant.

In Cold pressor test, the mean of Systolic BP was 121.55±8.283 for Group B and for Group A 119.75±7.334. The mean of diastolic BP was 79.75±4.79 for Group B and 78.00±6.485 for Group A. The mean values of systolic and diastolic BP were increased in Group B when compared with Group A but not statistically significant.

The mean and standard deviation value of QTc interval for Group B was 0.363±0.100 and for Group A 0.346±0.729. It was found to be statiscally significant (P=0.019).

Figure No.11 Parameters reflecting the Sympathetic activity

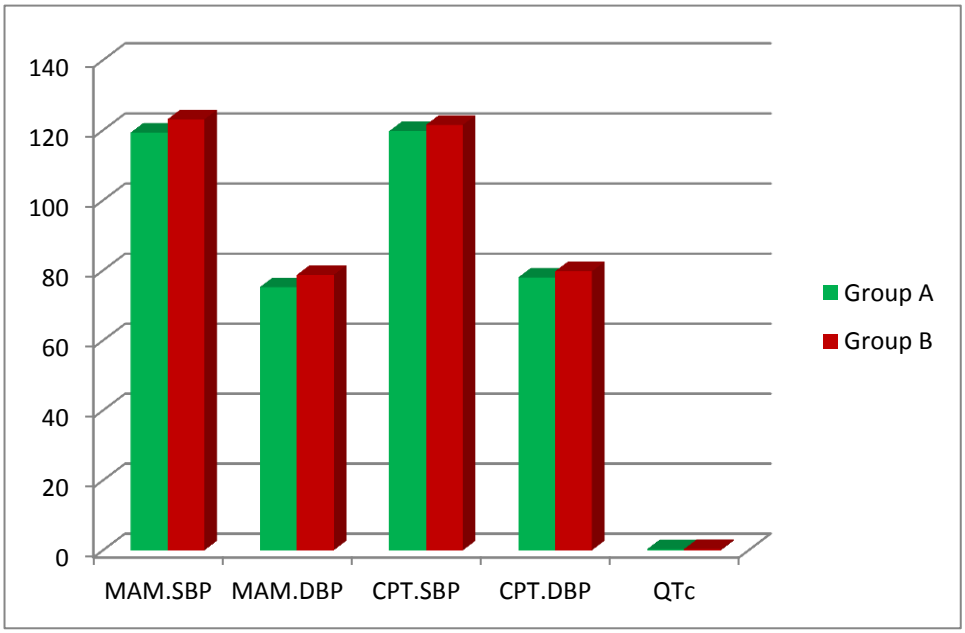


Table no:5

Comparison of Parasympathetic functions

Parameters	Group A n=40 Mean±SD	Group B n=40 Mean±SD	P value
Valsalva Ra.	1.22±0.07	1.08±0.11	< 0.001*
S / L Ra.	3.72±16.91	0.88±0.11	0.29
30 : 15 Ra.	1.06±0.07	0.96±0.10	< 0.001*
E : I Ra.	1.22±0.09	1.08±0.10	< 0.001*

The mean and standard deviation value of Valsalva ratio test for Group B was 1.08±0.11 and for Group A 1.22±0.07. It was found to be significantly reduced (P<0.001).

The mean and standard deviation value of Standing / lying ratio for Group B and Group A were 0.88±0.11 and 3.72±16.91 respectively. It was not found to be statistically significant (P=0.29).

The mean and standard deviation value of 30:15 ratio for Group B was 0.96±0.100 and for Group A 1.06±0.07. It was found to be statistically significant (P<0.001).

The mean and standard deviation value of E: I ratio for Group B and Group A were 1.08±0.10 and 1.22±0.09 respectively. It was found to be significantly reduced (P<0.001), thus reflecting decreased parasympathetic activity.

Figure No: 12

The parameters reflecting the Parasympathetic activity

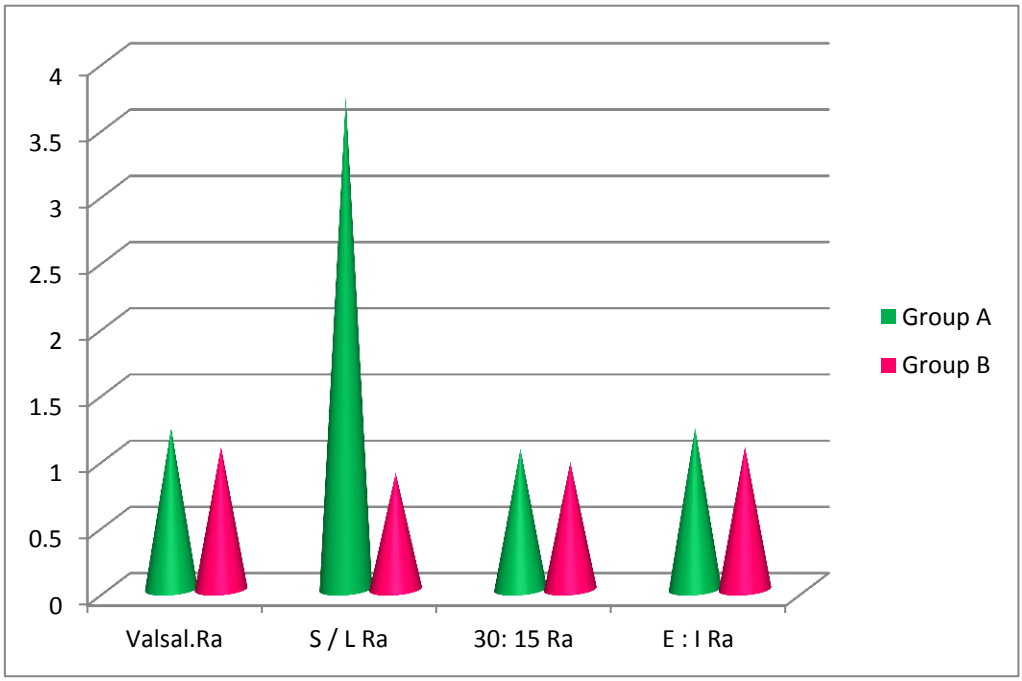


Table No:6

Comparison of Serum Estrogen levels in Group A and Group B

Estrogen pg/dl	Mean	S.D	P value
Group A (n=40)	94.3275	47.15459	< 0.001*
Group B (n=40)	46.0587	46.10235	

The mean and standard deviation value of estrogen in Group B was 46.05±46.10 and for Group A 94.32±47.15, which was found to be significantly reduced (P<0.001).

Figure No:13

Comparison of Sr.Estrogen levels in Group A and Group B

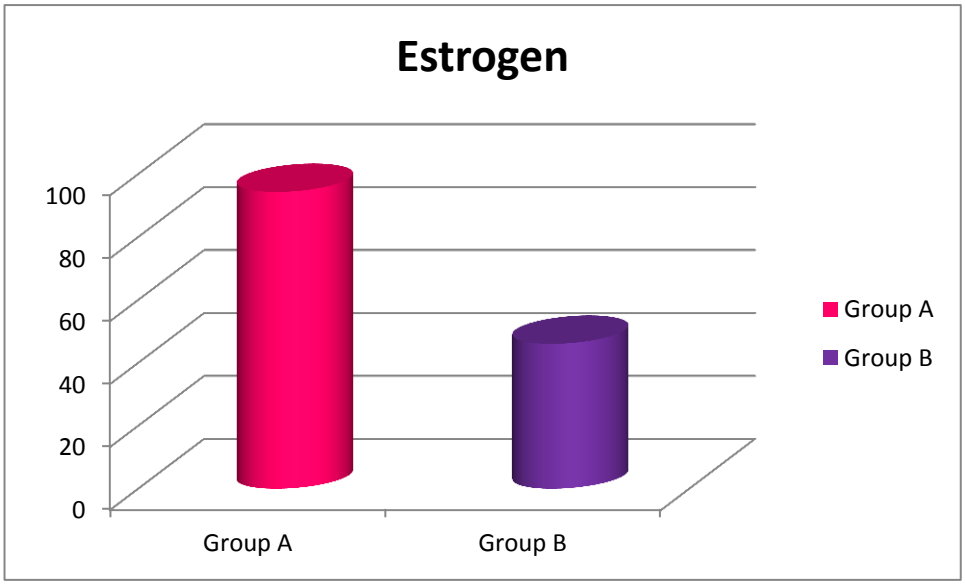


Table No:7

Correlation of Sr.Estrogen levels with Valsalva ratio in Group B

Parameter	Correlation value 'r'	P value
Valsalva Ratio	0.5857	0.001

Valsalva ratio showed positive correlation with serum estrogen level in Group B and the relation was found to be statistically significant.

Figure No:14

Correlation of Sr.Estrogen levels with Valsalva ratio in Group B

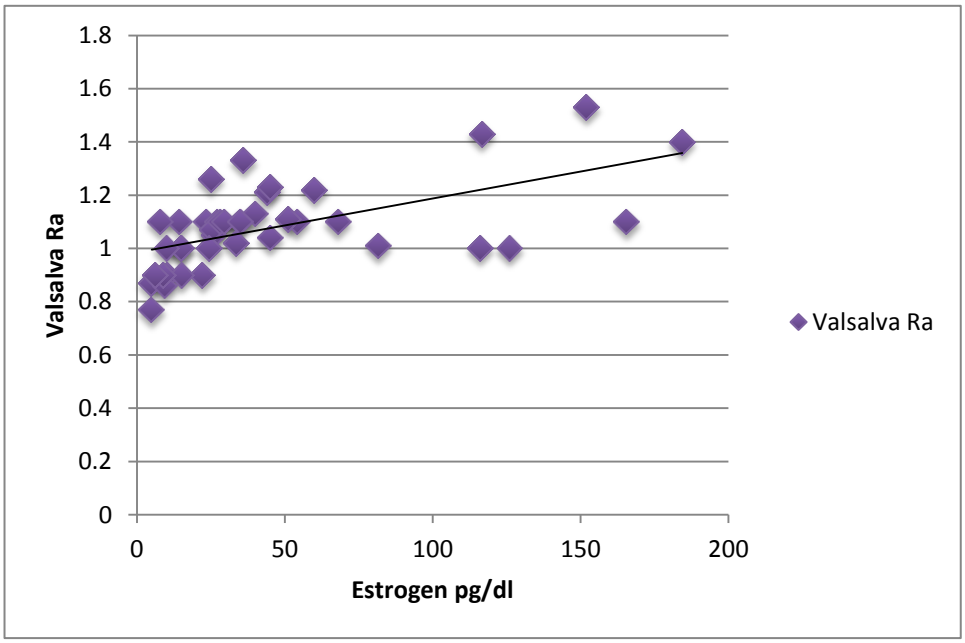


Table No:8

Correlation of Sr.Estrogen levels with Valsalva ratio in Group A

Parameter	Correlation value- 'r'	P value
Valsalva ratio	-0.0673	0.6

Valsalva ratio showed negative correlation with Sr.estrogen levels in Group A. It was found to be statistically insignificant.

Figure No: 15

Correlation of Sr.Estrogen levels with Valsalva ratio in Group A

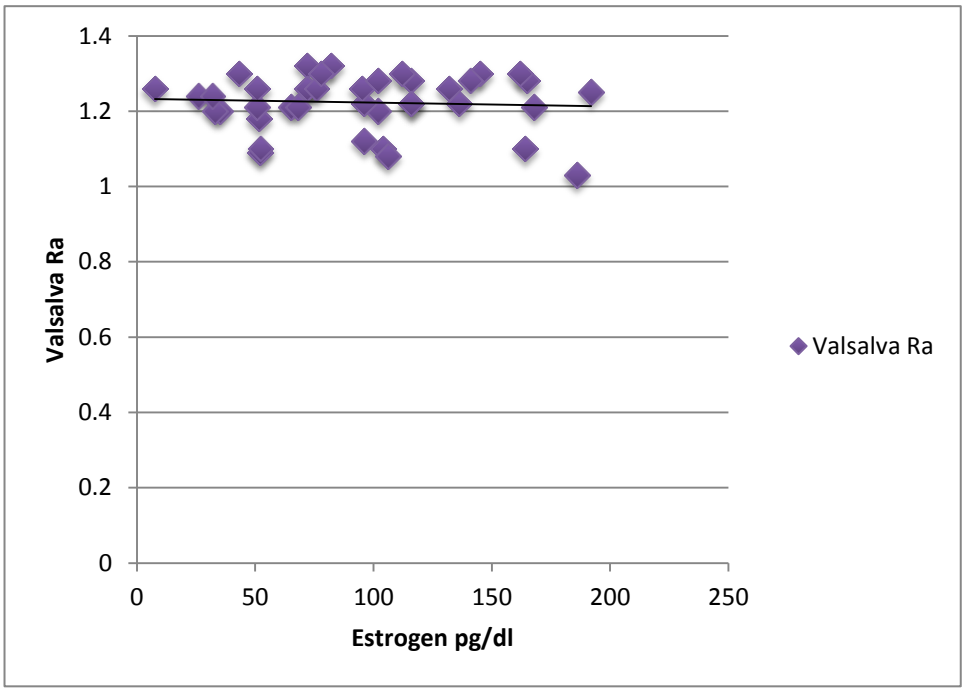


Table No:9

Correlation of Sr.Estrogen levels with E:I ratio in Group B

Parameter	Correlation value 'r'	P value
E:I Ratio	0.5429	0.0003

` E : I ratio was positively correlated with serum estrogen level in Group B and it was found to be statistically significant.

Figure No: 16

Correlation of Sr.Estrogen levels with E:I ratio in Group B

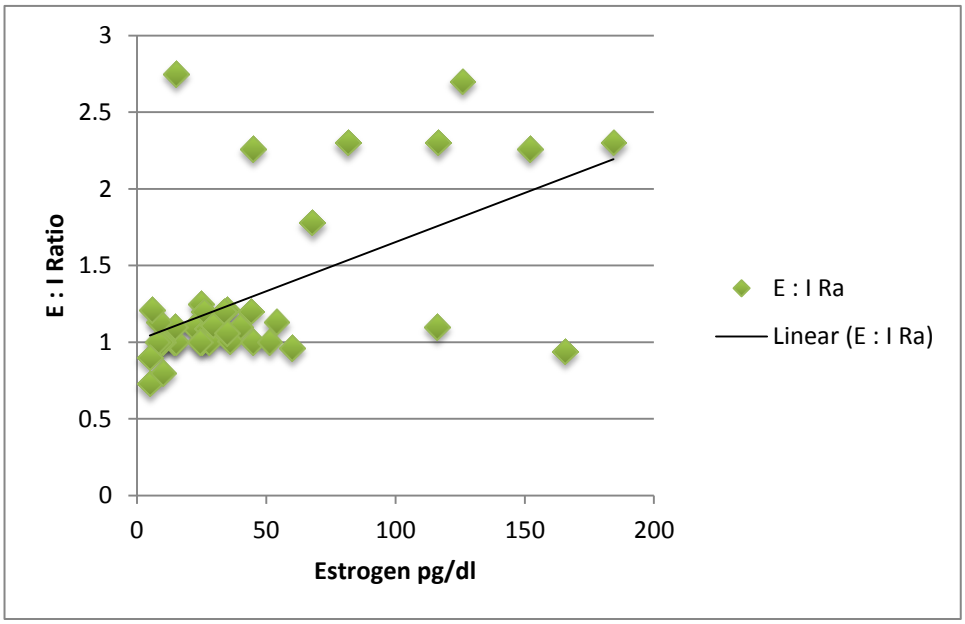


Table No:10

Correlation of Sr.Estrogen levels with E:I ratio in Group A

Parameter	Correlation value-'r'	P value
E:I ratio	-0.1561	0.3360

E:I ratio showed negative correlation with Sr.estrogen levels in Group A. It was found to be statistically insignificant.

Figure No: 17

Correlation of Sr.Estrogen levels with E:I ratio in Group A

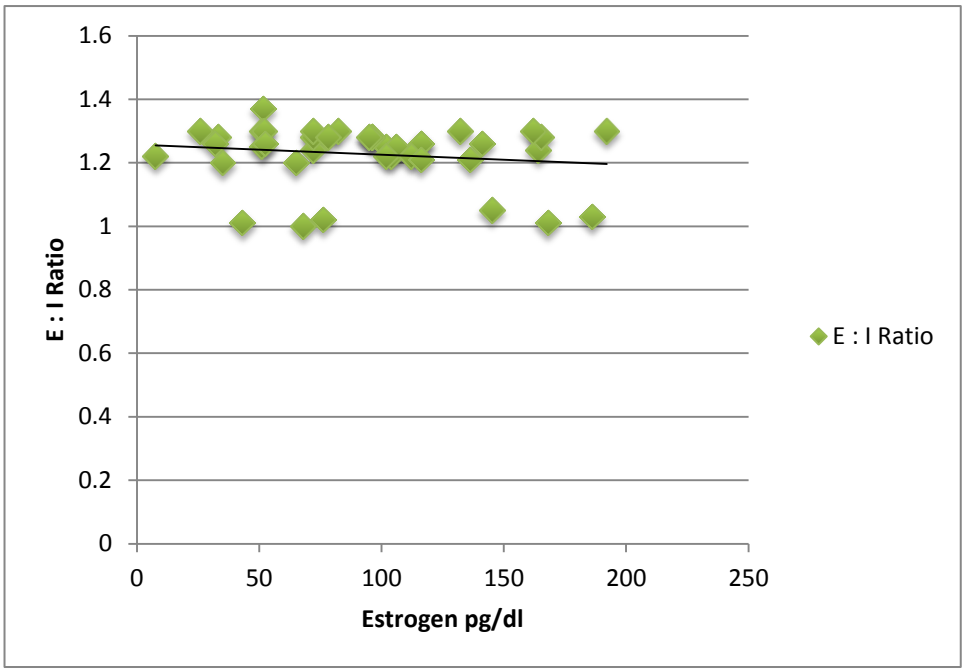


Table No:11

Correlation of Sr.Estrogen levels with 30:15 ratio in Group B

Parameter	Correlation value 'r'	P value
30:15 Ratio	0.3136	0.04

30:15 ratio was positively correlated with serum estrogen level in Group B and it was found to be statistically significant.

Figure No: 18

Correlation of Sr.Estrogen levels with 30:15 ratio in Group B

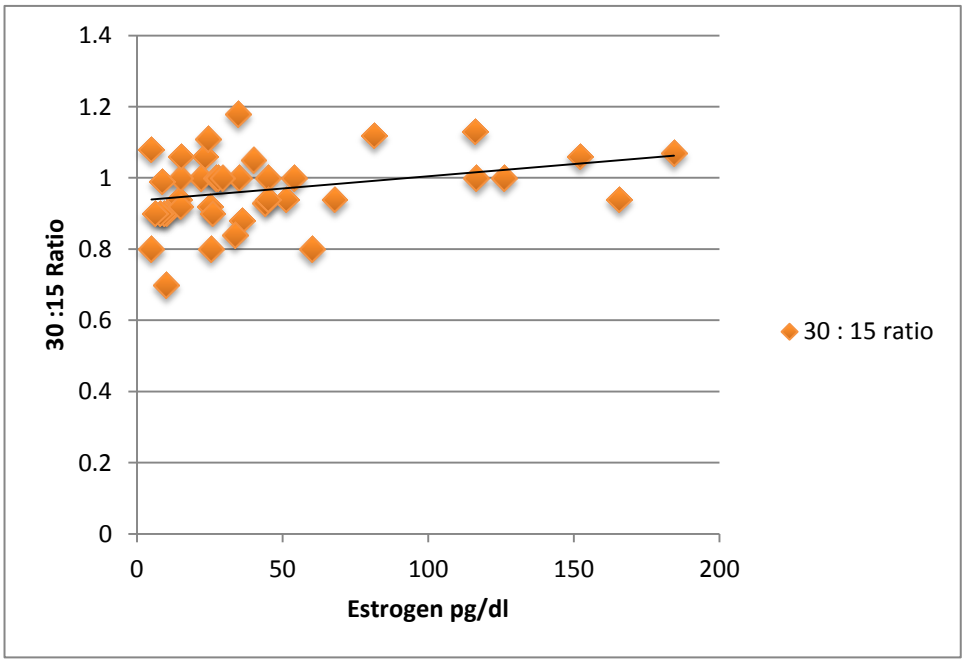


Table No:12

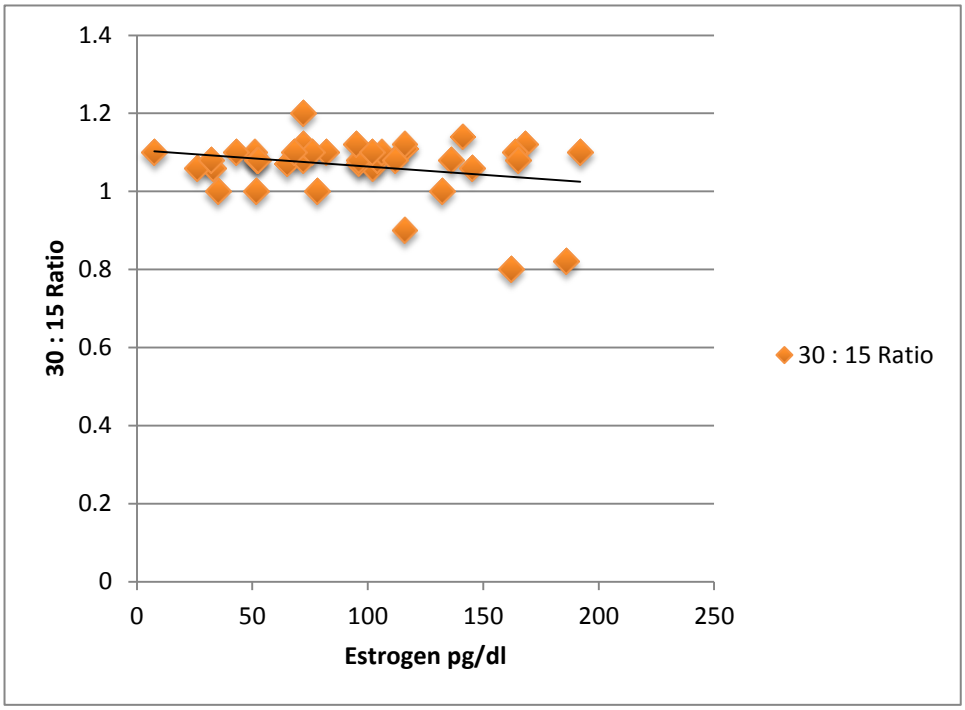
Correlation of Sr.Estrogen levels with 30:15 ratio Group A

Parameter	Correlation value 'r'	P value
30:15 Ratio	-0.2584	0.107

30:15 ratio showed negative correlation with Sr.estrogen levels in Group A. It was found to be statistically insignificant.

Figure No: 19

Correlation of Sr.Estrogen levels with 30:15 ratio in Group A



DISCUSSION

DISCUSSION

Menopause is a normal aging phenomenon in women. Perimenopause in life is a critical period during which endocrinological, psychological, and somatic alterations occur in the transition to menopause. The perimenopausal period includes the changes from the ovulatory cycle, up to the cessation of mensus. The serum estradiol levels do not decline until less than a year before menopause.

Menopause is the permanent cessation of mensus as a result of irreversible loss of a number of ovarian functions including ovulation and estrogen production. These women often suffer from various menopausal complications including autonomic nerve dysfunction. Postmenopausal women reported more vasomotor symptoms like hot flushes, sweating when compared with perimenopausal women.

In this study, Sr. estrogen levels of postmenopausal women showed significantly reduced values, when compared to the perimenopausal women. The present study has shown significant alteration in the autonomic function tests, which includes sympathetic as well as the parasympathetic functions. .

Sympathetic function tests:

Pulse rate and Blood Pressure

The mean ± SD of pulse rate for the postmenopausal women was (87.4±7.063), which is significantly higher than the perimenopausal women (P=0.001). It shows increased sympathetic activity.

Anjali Nadir Bhat et al have studied the autonomic functions in healthy postmenopausal women. Their results showed high significant variation in the pulse rate. The present study is congruent with this literature cited. Similar findings were observed in the previous studies [30, 43, 44,45].

The supine SBP were found to be significantly elevated in the postmenopausal women (120.65±7.25, P=0.004). The DBP was elevated when compared to perimenopausal women, though it was not statistically significant (77.10±5.728, P=0.07), which reflects the increased sympathetic activity.

Latifa Afrin Dill Naher et al., performed sympathetic function tests in postmenopausal women and their results showed increased resting SBP and DBP, which again reflects the increased sympathetic activity. The present study is also congruent with those found by Neves et al, Anjali Nadir Bhat et al, Shaher Lavi et al., This study also coincides with the previous study done by Ronald E. De meersman et al., who showed decreased systolic and diastolic blood pressure after estrogen replacement therapy [46].

Orthostasis:

The present study showed significant fall in systolic BP after standing from lying position, which indicates sympathetic hyperactivity in postmenopausal women.

Latifa Afrin Dill Naher et al observed significant fall in systolic BP after standing from lying position. The present study is congruent with this literature cited. Anjali Nadir Bhat et al also showed significant variation in orthostasis (SBP), which is similar to the present study. Paula J. Harvey et al studied the blood pressure responses to orthostasis in healthy postmenopausal women, reflecting the influence of estrogen through renin angiotensin system [47].

Cold Pressor Test:

In the present study, Cold pressor test (SBP, DBP) showed increased mean values, thus indicating an increase in the sympathetic activity, though it was not statistiscally significant (SBP-121.55±8.283, P=0.3 and DBP-79.75±4.797, P=0.17).

L.Mouret et al showed CPT triggers an increase in blood pressure in the healthy subjects, that may be due to increased cardiac output and increase in muscle sympathetic nerve activity [48]. Similar findings were observed in the previous studies by Victor RG et al [49].

Mental Arithmetic Mean:

The present study showed increased SBP and DBP of Mental Arithmetic Mean in the postmenopausal women, when compared to the perimenopausal women, which was found to be statistically significant(SBP-123.15±6.912,P=0.01 and DBP-78.75±4.043,P=0.003). It reflects the increase in sympathetic activity.

Mi Kyong PARK et al observed increased blood pressure of MAM in postmenopausal and perimenopausal women due to vasoconstriction caused by the sympathetic activation. The present study is congruent with this literature cited and also in accordance with the study done by Anjali Nadir Bhat et al. Jason R.carter et al studied the sympathetic neural responses to mental stress in humans, which showed elevated mean arterial pressure [(50)].

QTc Interval:

In the present study, the mean±SD of QTc interval showed significant variation in the postmenopausal women (0.36±0.1, P=0.019), which indicates the increased sympathetic activity.

ArDuino A.et al studied the relationship between age and QT interval. Their results showed prolongation of QT interval with advancing age. They explained it may be due to the changes observed in the heart and vasculature of the healthy elderly subjects. These include cardiac hypertrophy, increased vascular stiffness and aortic impedance. The cardiac hypertrophy is due to an increase in size of cardiac myocytes and is associated with a significant

prolongation of the transmembrane action potential. The present study is also in accordance with the Reardon et al [51], Taneja et al [52], and Pham TV et al [53].

Parasympathetic function tests:

Valsalva Ratio:

In the present study, mean and SD of the Valsalva ratio showed reduced values, which is statistically significant (1.0835±0.11). Thus, it indicates the decreased parasympathetic activity.

G.V.Lathadevi et al., showed reduced values of valsalva ratio. The present study is congruent with this literature cited. Valsalva ratio was positively correlated with Sr.estrogen level in the postmenopausal women, which showed statistically significant relation. These findings were in accordance with Naher LAD et al [54].

Expiration: Inspiration Ratio:

The mean and SD of E: I ratio in postmenopausal women were found to be decreased, which reflects the reduced parasympathetic activity (1.0805±0.10, $P<0.001$). E:I ratio also showed significant positive correlation with estrogen level in postmenopausal women, that indicates parasympathetic nerve functions, which are associated with decreased level of estrogen in them. Naher LAD et al observed similar findings in postmenopausal women. Similar findings were observed in previous studies by Virtanen et al [55], M.Zi et al [56].

Standing / Lying ratio:

In the present study, mean and SD of Standing / Lying ratio were found to be reduced, though it as not statistically significant (0.88±0.11, P=0.29).

Anjali Nadir Bhat et al also observed the variation in S/L ratio, which was statistically insignificant. G.V.Lathadevi et al showed reduced values of S/L ratio that was not statistically significant. The present study is in accordance with the literature cited.

30:15 Ratio:

In this present study, the mean and SD of 30:15 ratio in the postmenopausal women showed significantly reduced values (0.9675±0.100, P<0.001). It also showed significant positive correlation with Sr.estrogen level in postmenopausal women (P=0.004). Naher et al observed significant variation in postmenopausal and also showed positive correlation with Sr.estrogen level in postmenopausal women, reflecting reduced parasympathetic activity.

Estrogen:

Estrogens are believed to be potent regulators of mind and mood. The present study tested the hypothesis that estrogen exerts regulatory influence on ANS in postmenopausal women.

In the present study, mean and SD of Sr.estrogen in postmenopausal women was significantly lowered when compared to perimenopausal women (46.05±46.102, P<0.001). Latifa Afrin Dill Naher et al showed significantly reduced levels of estrogen in postmenopausal women. They also demonstrated the increased sympathetic activity and decreased parasympathetic activity in postmenopausal women that was related to low estrogen level.

Estrogen has a sympatho-inhibitory effect. Estrogens increases density as well as the function of presynaptic α2 adrenoreceptors, thereby resulting in significant decrease of nor-epinephrine induced responses. So, estrogen deficiency in postmenopausal women may lead to increased basal level of NE and its vasoconstrictor responses [57,58,59].

Estrogen also acts peripherally to increase vasodilatation by increasing the production of Nitric oxide and prostacycline or by decreasing the release of endothelin from endothelium [60]. It has direct effect on vascular smooth muscle to cause muscle dilatation [61].

Parameters reflecting sympathetic activity like pulse rate, Blood pressure, MAM, QTc interval showed a greater proportion in postmenopausal women when compared with perimenopausal women which was statistically significant.

Parameters reflecting parasympathetic activity like valsalva ratio, E:I ratio, 30:15 ratio were reduced in postmenopausal women when compared with perimenopausal women which was statistically significant.

CONCLUSION

CONCLUSION

The present study shows significant alteration in the Autonomic functions of postmenopausal women when compared with perimenopausal women.

In this study, the result shows increased sympathetic and reduced parasympathetic activity in postmenopausal women.

Parameters reflecting parasympathetic activity like valsalva ratio, 30:15 ratio, E:I ratio shows positive correlation with serum estrogen level in postmenopausal women.

Estrogen levels are reduced in the postmenopausal women, when compared with perimenopausal women that exert a regulatory influence on autonomic functions**.**

Cold pressor test reflecting sympathetic activity does not show significant increase in perimenopausal women.

Standing / lying ratio reflecting parasympathetic activity does not show significant decrease in perimenopausal women.

Assessment of sympathetic and parasympathetic functions, could therefore aid in early diagnosis of Autonomic dysfunction in postmenopausal women. Hence, estrogen may be administered early in them, which would prevent the cardiovascular complications.

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BIBLIOGRAPHY

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ANNEXURES

CONSENT FORM

Dr. M.Vijayalakshmi post graduate student in the Department of physiology, Thanjavur Medical College, Thanjavur is doing a comparative study on autonomic functions in perimenopausal women and postmenopausal women. The procedures have been explained to me clearly. I understand that there are no risks involved in the above procedure. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature:

Name:

Place:

PROFORMA

TOPIC : Comparison of Autonomic functions in Perimenopausal women with Postmenopausal women.

Name of the Patient / Control:

AGE : SEX :

ADDRESS : OCCUPATION :

PRESENT H/O	:	Hot flushes /Sweating /Headache / Dizziness.	
PAST H/O	:	Diabetic / HT / CVS disorders / Renal / Liver diseases / Surgical menopause	
PERSONAL H/O	:	Smoking / Alcohol	
TREATMENT H/O	:	Oral Pills / HRT.	
FAMILY H/O	**:**		
MENSTRAL H/O	:	RMP / IRMP :	FMP :
		No. of Cycles /Month :	LMP :
		Mother's age of menopause :	

GENERAL EXAMINATION / VITAL SIGNS :

ANEMIA	Ht :	Wt :
CYANOSIS		
CLUBBING		
PEDAL EDEMA	:	
JAUNDICE	:	
LYMPHADENOPATHY		
PR :	BP :	RR :
Examination of CVS	**:**	
Examination of RS	**:**	
Examination of CNS	**:**	
Examination of Abdomen	**:**	

SPECIAL INVESTIGATIONS :

SYMPATHETIC FUNCTION TESTS :

PULSE RATE	
BP	
COLD PRESSOR TEST	
ORTHOSTASIS	
MENTAL ARITHMETIC MEAN	
QT INTERVAL	

PARASYMPATHETIC FUNCTION TESTS

VALSALVA MANAVEOUR	
EXP / INS RATIO	
STANDIG / LYING RATIO	
30 :15 RATIO	

BIOCHEMICAL ANALYSIS :

ROUTINE INVESTIGATION :

(1). URINE - ALBUMIN **:**

- SUGAR **:**

(2). RANDOM BLOOD SUGAR :

(3). Hb %

SPECIAL INVESTIGATION :

(1). ESTROGEN :

(CLIA)

ABBREVIATIONS USED IN THIS STUDY

ANS	-	Autonomic Nervous System
SBP	-	Systolic Blood Pressure
DBP	-	Diastolic Blood Pressure
CPT	-	Cold Pressor Test
MAM	-	Mean Arithmetic Mean
QTc Interval	-	Corrected QT Interval
Val Ra	-	Valsalva Ratio
E:I Ra	-	Expiration Ratio
S/L Ra	-	Standing / Lying Ratio
HRR	-	Heart Rate Response
GnRH	-	Gonadotrophin Releasing Hormone
FSH	-	Follicle Stimulating Hormone
LH	-	Luteinizing Hormone
NTS	-	Nucleus Tractus Solitarious
FMP	-	First Menstrual Period
LMP	-	Last Menstrual Period

AUTONOMIC FUNCTION TESTS FOR GROUP B

S.No	Age	PR	Supine BP		Orthostasis		Ort.va	MAM		CPT		QTc	Val.Ra	S:L Ra	30:15 Ra	E:I Ra	Est pg/dl	Bld. sugar mgs/dl
			SBP	DBP	SBP	DBP		SBP	DBP	SBP	DBP							
1	50	96	110	80	90	70	20	114	80	110	80	0.29	1.21	0.9	0.93	1.2	44	100
2	55	90	116	80	90	70	26	114	80	110	80	0.43	1.4	1.2	1.07	2.3	184.4	80
3	52	100	120	80	110	70	10	120	80	130	80	0.27	1.1	0.84	1.06	1.1	23.42	88
4	51	90	124	80	110	72	14	130	80	130	90	0.52	0.9	0.78	1	1.1	22.2	85
5	50	88	128	70	102	60	26	130	70	130	80	0.3	1.1	0.79	0.94	0.94	165.6	77
6	55	94	120	80	90	70	20	130	80	120	80	0.56	1.26	0.8	0.92	1.25	25.02	89
7	54	102	130	80	106	76	24	130	80	130	80	0.49	1.1	0.7	1	1.09	27.26	96
8	55	86	130	70	110	60	20	130	70	130	80	0.31	1.05	0.9	0.9	1.2	26	84
9	55	88	120	84	102	60	18	120	90	126	80	0.27	1.43	0.9	1	2.3	116.6	114
10	50	90	120	70	120	70	0	120	70	120	80	0.45	1.07	0.86	0.8	1	25.4	100
11	55	82	120	60	110	60	10	120	70	120	70	0.3	1.13	0.9	1.05	1.1	40.1	110
12	53	86	134	80	110	70	24	140	80	130	80	0.5	1.1	0.94	0.94	1	14.36	117
13	55	84	128	60	110	70	26	140	80	120	80	0.34	0.87	0.9	1.08	0.73	5	100
14	46	86	120	80	100	70	20	120	80	120	80	0.23	1	0.94	1	2.7	126	40.5
15	48	84	120	80	102	70	18	126	80	130	80	0.32	1.1	0.96	1	1.21	34.98	82
16	49	80	130	80	108	70	22	130	80	130	80	0.36	1.1	0.84	1	1	28.05	86
17	54	82	120	80	104	70	16	120	80	120	80	0.3	1.1	1.1	1	1.11	29.38	90
18	47	90	110	80	110	80	0	116	80	110	80	0.27	1.33	0.89	0.88	1.01	36.02	110
19	49	86	120	80	102	70	18	120	80	120	80	0.27	1.23	0.88	1	1	45	68

S.No	Age	PR	Supine BP		Orthostasis		Ort.va	MAM		CPT		QTc	Val.Ra	S:L Ra	30:15 Ra	E:I Ra	Est pg/dl	Bld. sugar mgs/dl
			SBP	DBP	SBP	DBP		SBP	DBP	SBP	DBP							
20	50	84	116	80	90	70	26	120	80	120	80	0.31	1	0.87	1	1	15.05	74
21	53	76	120	80	100	70	20	120	80	120	80	0.48	1.1	0.94	1.18	1.06	34.8	98
22	50	74	130	80	110	70	20	130	80	130	80	0.33	1	0.86	1.13	1.1	116.05	96
23	55	76	120	80	120	80	0	120	80	126	90	0.32	1.1	0.83	1	1.13	54.09	104
24	50	80	130	80	100	70	20	130	80	130	80	0.29	1.01	0.86	1.12	2.3	81.52	96
25	53	82	120	80	102	70	18	120	80	120	80	0.55	1.11	0.95	0.94	1	51.32	110
26	46	86	130	80	100	70	30	130	80	130	90	0.51	1.53	0.63	1.06	2.26	152.02	81
27	50	88	128	80	100	70	28	130	80	130	80	0.27	0.9	0.89	0.9	1	10.16	98
28	55	108	116	80	110	80	6	120	80	120	80	0.27	1	0.9	1.11	1	24.54	96
29	50	94	120	80	100	70	20	120	80	130	90	0.29	0.9	0.76	1.06	2.75	15.15	112
30	50	98	110	70	90	70	20	120	80	120	80	0.33	0.86	0.89	0.9	1	9.37	98
31	53	90	110	70	110	70	0	120	80	120	80	0.33	1.04	1.05	0.94	2.26	45.02	102
32	55	94	120	80	100	70	20	126	80	100	70	0.297	1	0.78	0.92	1.1	15	120
33	52	88	110	70	110	70	0	110	70	110	70	0.297	1.1	0.73	0.9	1	7.97	96
34	55	86	100	70	90	70	10	110	70	100	70	0.31	0.77	0.94	0.8	0.9	5	100
35	50	84	120	80	100	70	20	120	80	110	70	0.33	0.9	0.94	0.99	1.13	8.77	110
36	55	82	120	80	102	70	18	120	80	120	80	0.29	1.1	0.7	0.94	1.78	67.92	95
37	48	80	130	80	110	70	20	130	80	130	80	0.34	1	0.87	0.7	0.8	10.08	94
38	53	90	120	80	102	70	18	120	80	120	80	0.53	1.02	0.78	0.84	1.2	33.6	83
39	52	84	116	70	90	70	26	120	80	120	80	0.55	0.9	1.08	0.9	1.21	6.08	102
40	55	88	120	80	100	70	20	120	80	120	80	0.52	1.22	1.08	0.8	0.96	60.05	96

AUTONOMIC FUNCTION TESTS FOR GROUP A

S.No	Age	PR	Supine BP		Orthostasis		Ort. Var	MAM		CPT		QTc	Valsal Ra	S:L Ra	30:15 Ra	E:I Ra	Estrogen pg/dl	Bld sugar mgs/dl
			SBP	DBP	SBP	DBP		SBP	DBP	SBP	DBP							
1	45	76	100	70	100	70	0	110	70	110	70	0.33	1.2	1	1	1.2	35.06	72
2	48	82	110	70	110	70	0	118	70	120	70	0.32	1.26	0.8	1.1	1.22	7.56	93
3	50	82	126	80	120	80	6	130	80	130	90	0.27	1.18	1.38	1	1.37	51.69	116
4	51	72	100	70	100	70	0	110	70	110	80	0.49	1.2	1.06	1.06	1.25	102.06	106
5	50	70	100	70	100	70	0	110	70	110	70	0.39	1.21	0.89	1.07	1.2	65.02	102
6	46	78	100	70	100	70	0	110	70	110	80	0.33	1.22	1.2	1.11	1.26	116.08	98
7	47	72	126	80	100	70	26	130	80	130	90	0.3	1.22	0.88	1.08	1.21	136.28	80
8	45	84	100	90	100	80	0	110	70	110	70	0.31	1.22	1.2	1.07	1.28	96.02	102
9	45	84	126	70	100	60	26	130	80	130	90	0.2	1.3	1.06	1.06	1.05	145.06	86
10	49	90	110	70	110	70	0	120	70	120	70	0.3	1.03	1.02	0.82	1.03	186.03	84
11	53	86	116	70	90	70	26	120	70	120	70	0.42	1.32	0.7	1.08	1.24	72.05	102
12	50	74	116	80	110	70	6	120	80	120	80	0.28	1.1	108	1.08	1.22	104.06	106
13	52	84	120	80	102	70	18	120	80	130	90	0.29	1.21	1.1	1.09	1.25	51.02	72
14	51	88	112	80	110	80	2	120	90	120	80	0.3	1.25	0.8	1.1	1.3	192.05	100
15	50	82	114	60	110	60	4	120	80	120	70	0.25	1.26	1.09	1.12	1.28	72	90
16	48	84	120	80	90	70	30	120	90	120	80	0.4	1.08	1.1	1.1	1.25	106.06	88
17	51	80	100	70	100	70	0	110	70	110	70	0.46	1.2	1.06	1.06	1.28	33.03	74

S.No	Age	PR	Supine BP		Orthostasis		Ort. var	MAM		CPT		QTc	Valsal Ra	S:L Ra	30:15 Ra	E:I Ra	Estrogen pg/dl	Bld sugar mgs/dl
			SBP	DBP	SBP	DBP		SBP	DBP	SBP	DBP							
18	49	82	100	70	100	70	0	110	70	110	70	0.45	1.32	0.9	1.1	1.3	82.08	104
19	53	88	130	80	100	70	30	130	80	130	80	0.38	1.26	1.02	1.1	1.3	51.03	84
20	48	90	120	80	120	80	0	120	80	120	80	0.35	1.21	1.08	1.12	1.01	168	96
21	52	92	130	80	130	80	0	130	80	130	80	0.29	1.12	1.1	1.08	1.28	96.04	106
22	50	94	130	70	110	70	20	130	70	130	80	0.3	1.28	1.2	1.12	1.26	116.02	86
23	49	88	130	80	130	80	0	130	80	130	90	0.5	1.1	1.04	1.1	1.24	164	76
24	51	82	130	70	130	70	0	130	70	130	80	0.49	1.3	1.1	1.08	1.22	112	104
25	46	76	120	80	120	80	0	130	70	130	80	0.32	1.26	1.06	1.2	1.3	72.05	96
26	48	68	116	70	110	70	6	120	70	120	80	0.42	1.22	1.06	0.9	1.21	116.02	96
27	51	82	100	70	100	70	0	110	70	110	70	0.5	1.26	1.04	1.1	1.02	76.05	130
28	46	84	116	80	110	70	6	120	80	120	80	0.4	1.28	1.1	1.08	1.28	165.02	116
29	49	86	120	80	90	70	30	120	80	120	80	0.37	1.24	0.9	1.06	1.3	26.05	104
30	52	92	116	70	110	70	6	120	70	120	80	0.42	1.3	1.06	1.1	1.01	43.08	110
31	53	94	120	80	90	70	30	120	80	120	80	0.4	1.28	1.04	1.14	1.26	141.02	120
32	48	84	110	70	110	70	0	120	70	120	70	0.32	1.09	1.1	1.08	1.3	52.03	130
33	51	70	116	70	110	70	6	120	70	120	80	0.33	1.21	1.02	1.1	1	68.04	110
34	45	76	120	80	90	70	30	120	80	120	80	0.3	1.26	1.08	1.12	1.28	95.02	96
35	50	78	110	70	110	70	0	116	70	120	80	0.31	1.3	1.18	0.8	1.3	162	84
36	49	68	116	80	110	80	6	120	80	120	80	0.38	1.28	1.21	1.1	1.22	102	90
37	53	70	110	70	110	70	0	110	70	110	70	0.4	1.24	1.1	1.08	1.26	32.05	100
38	46	80	120	80	92	70	28	120	80	120	80	0.32	1.26	1.04	1	1.3	132.05	106
39	52	92	110	80	110	80	0	110	80	110	80	0.3	1.3	1.1	1	1.28	78.02	120
40	47	74	110	70	110	70	0	110	70	110	70	0.35	1.1	1.06	1.08	1.26	52.3	100